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Review

Using green engineered nanoparticles for plant health management

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Nanoparticles are considered as building block element for the science nanotechnology. Nanotechnology offers feasible and most efficient alternative to scale up the agricultural production without hampering the environmental health. In agricultural science, nanotechnology can be used for early detection and diagnosis of various pests, diseases, disorders, nutrient deficiencies and pesticide residues, thus allows timely and appropriate usage of corrective measures. It also aids in monitoring various environmental stresses and soil health status. Besides, nanoparticles can be used as antifungal, antibacterial and antiviral agents. Furthermore, nanoparticles can be used for drug delivery systems for target delivery of agrochemicals, plant hormones and genetic materials. Studies revealed green engineered nanoparticle like Ag, Cu, Au, ZnO etc can be potentially used for management of plant heath with increased concentration of major secondary metabolites in the treated plants.

Keywords : Disease management, nanoparticles, nanotechnology, secondary metabolite

INTRODUCTION

Nanotechnology is an interdisciplinary science and has wide range of applications including physics, chemistry, biology, chemical engineering, electrical engineering, biophysics, biochemistry, biotechnology etc. During the last few decades, development of physics in areas such as nanoelectronics, nanomechanics, nanophotonics and nanoionics has led to the development of basic foundation of the science nanotechnology. Bionanotechnology, is a branch of nanotechnology, which includes conjunction between biotechnology and nanotechnology for developing biosynthetic and environment friendly technologies for the synthesis of various nanomaterials (Sobha *et al*. 2010). Multiple research regarding synthesis of nanoparticles has put forth great interest towards this novel science due to their unique physical and chemical properties than its macroscopic counterpart (Sau *et al*. 2010).

Nanotechnology has diverse field of applications which includes disease diagnostics, drug delivery, disinfectants, sunscreen, cosmetics, and food products. Silver nanoparticles are used in food packaging, fabrics, and various domestic appliances such as silver nano and carbon nanotubes for stain-resistant textiles, cerium oxide as a fuel catalyst.

Nanotechnology is also extensively used in food industry for packaging food materials. It is useful in developing antimicrobial packaging of food products. Nanoparticles are uniformly dispersed throughout the food packets which are capable of blocking oxygen, carbon dioxide and moisture from reaching fresh foods. Packaging helps to control microbial growth in food stuffs and thus prevents spoiling of food and spread of infectious diseases (Rajamanickam *et al.* 2012). Nanotechnology is also being applied for purification and bioremediation processes. Environmental purification applications include desalination of water, waste water treatment, water filtration, ground water treatment and other nanoremediation. In industries, nanoparticles are used in construction materials, military goods,

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manufacturing of nanowires, nanorods, few layers of graphene (Jayasena *et al*. 2011). The recent field of nanobiotechnology also offers tremendous applications which includes potential use of nanofungicides in agriculture, in anticancer drug and in imaging biomedical applications (Sharma *et al.* 2012).

Nanoparticles

A nanoparticle can be defined as the particle of matter having diameter in the range 1 to 100 nm. IUPAC defined nanoparticle as "a particle of any shape with dimension in the 1x 10^{-9} and 1x 10^{-7} m range"(Vert *et al.* 2012). Nanoparticles being at the transition between bulk phase and atomic and molecular structures, often exhibits unique phenomena that are not observed at either scale (Choi *et al.* 2008; Khanna *et al*. 2016). The production of nanoparticles with desirable properties is an important branch of nanotechnology. Nanoparticles exhibit a great variety of structure and morphologies such as nanorods, nanochains (Kralj *et al*. 2007) nanospheres (Agam *et al*. 2007), nanoflowers, nanostars, nanoreefs (Choy *et al*. 2004), nanofibers, nanowhiskers and nanoboxes (Sun *et al*. 2002). Shape and size of nanoparticles are determined by the intrinsic habit of the crystal material, influence of environment around their creation, shape of emulsion droplets and micelles in the precursor preparation, or the shape of pores surrounding the solid matrix (Murphy *et al*. 2002). Nanoparticles are prepared from materials having diverse chemical and physical nature , the most common being metals, metal oxides, non-oxide ceramics, polymers, silicates, organics, carbon and biomolecules.

Nanoparticles synthesized by various chemical and physical methods shows poor morphology. These methods and processes utilizes toxic chemicals and elevated temperature which poses serious threat to the environment (Sunkaworn *et al*. 2007; Rai *et al*. 2008). Metal oxides which acts common reducing agent for synthesis of nanoparticles, also serves as sorbents for various environmental pollutants. These challenges can be overcome by biological methods of nanoparticle synthesis using various microorganisms, plant extracts and enzymes. The biocompatibility of nanoparticles is more essential for biomedical application and researches (Sasidharan *et al*. 2013).

Commendable efforts have been made to explore antimicrobial property of silver nanoparticles against human pathogens, but insignificant research has been done to study its effects against overwhelming phytopathogens. Few studies have established the fungicidal effects of nanosilver against several phytopathogenic fungi (Kim *et al.*2012), but the fungicidal mechanism of this compound has not been clearly elucidated. There are volumes of literature stating the *in vitro* activity of AgNPs against several fungi, but very few researchers have paid attention to the application of AgNPs as antifungal compounds in controlling plant diseases in the fields and in turn promote the overall plant growth. Application of AgNPs in soil and as seed/seedling coatings may not only control the phytopathogen, but also stimulate plant growth by several known and unknown mechanisms.

Types of nanoparticles

Nanoparticles can be broadly grouped into two-namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi-conductor nanoparticles (like titanium oxide and zinc oxide, copper). There is a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility.

In plant pathology nanotechnology has versatile potential in the following aspects

1. **Detection of plant pathogen:** For detection and diagnosis of the causal factor of plant disease is possible through the use of different techniques of nanotechnology. Out of which, Quantum Dots, Gold nanoparticle, barcodeed DNA and nanobiosensors for detection of contaminated microbes in foods, plant system, mycotoxions get momentum in this direction.

2. **Plant disease management:** For plant disease management nanoparticele can be used

as antimicrobial agents i.e., both antifungal and antibacterial, nanosized silica-silver can be used for development of resistance in the plant with antimicrobial properties, nano particle like nanocapsules can be used to deliver the pesticides in agro-ecosystem, and plant disease forecasting, further nanoclays and nanoflims can be used as barrier materials to prevent fruit spoilage anti post harvest disease.

Synthesis of Metallic Nanoparticles

a. **Bottom-up approach:** The bottom-up approach is a nano-architectural phenomenon of self assembly of materials from cluster-to-cluster, molecule-to-molecule or atom-to-atom on top of a base substrate. The main concern in the bottom-up approach is the adhesion of the surface layers to the base substrate. The most commonly used bottom-up methods are wielding & riveting.

b.**Top down approach:** The Top down method refers to a set of fabrication technologies starting with a block bulk material which share the same material with the base substrate. The most commonly used top down methods are milling, drilling and grinding.

c.**Physical approach:** In physical approach metal nanoparticles are synthesised by either evaporation - condensation method or laser ablation method. In evaporation condensation method the reaction is carried out using a tube furnace at atmospheric pressure. The target material is kept within a boat centred at the furnace is vaporized into a carrier gas.

d. **Chemical approaches:** The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethyl formamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag+) in aqueous or non-aqueous solutions. It is important to use protective agents to stabilize dispersive nanoparticles during the course of metal nanoparticles preparation and protect the nanoparticles that can be absorbed on or bind onto nanoparticles surfaces, avoiding their agglomeration (Oliveira *et al.* 2005). The presence of surfactants comprising functionalities (*e.g.,* thiols, amines, acids, and alcohols) for interactions with particle surfaces can stabilize particle growth, and protect particles from sedimentation, agglomeration, or losing their surface properties.

Why need of Green Synthesis?

Although chemical and physical methods are very successful to produce well- defined nanoparticles, they have certain limitations such as increase cost of production, release of hazardous by-products, long time for synthesis and difficulty in purification (Nagajyothi and Lee, 2011). Global warming and climate change has induced a worldwide awareness to reduce the toxic & hazardous waste materials, thus, the green synthesis route have raised actively the progress in the fields of science and industry (Ahmad *et al.* 2011). Therefore, biological sources such as bacteria, fungi, yeasts, algae and plants can materials catalyzed specific reaction as a part of modern and realistic biosynthetic strategy. The current prospective on biological system has created a commercial importance due to their enzymatic reactions, photochemical characteristics and herbal nature.

The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by-products. Thus, there is an increasing demand for “green nanotechnology”. Microorganisms, whole plants, plant tissues and fruits, plant extracts and marine algae have been used to produces nanoparticles (Luangpipat *et al.* 2011). Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms (Singhal *et al.* 2011). Synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites (Ankamwar *et al.* 2005).

Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles (Kumar and Yadav, 2009). The source of the plant extract is known to influence the characteristics of the nanoparticles.

This is because different extracts contain different concentrations and combinations of organic reducing agents (Mukunthan and Balaji, 2012).

Green engineered nanoparticle

Green engineered nanoparticle has emerged as a new method for metal nanoparticle. Many fungal species like *Trichoderma*, *Agaricus*, *Fusarium*, *Aspergillus*, *Verticillium*, *Penicillium* are being used for green synthesis of metal nanopaticle like Silver, Zinc, Zirconia , Silica, Magnetite, Platinum, Gold, Cobalt, CdTe etc (Alghuthayami *et al.* 2015). These may be produced by the fungal species extra or intracellularly (Table 1). Though this is a challenging task, but was possible as we could able to produce Silver nanoparticle of <30 nm from *Trichoderma asperellum*. Reduction of silver ion is reflected in the colour of the cell filtrates, which vary from pale yellow to brown. The reason of using fungi for green synthesis of nanoparticle is due the fact that they have ability to produce large quantity of enzyme, and are easy to handle (Mandal *et al*. 2006; Mohanpuria *et al.* 2007). Moreover, nanoparticles produced by fungal species are easy to purify as they are produced extracellularly and can be used for different applications (Mukharjee *et al.* 2008; Gaikwad *et al.* 2013). Besides, these fungal mycelia mesh can withstand flow pressure and other conditions in bioreactors or other chambers as compared to plant material or bacteria (Narayan *et al.* 2010). Recently, by using *Trichoderma* spp. silver nanoparticle has been isolated. The nanoparticle was characterized by UV VIS spectrophotometer, Dynamic Light Scattering, Zetasizer, and Scanning Electron Microscopy and found the size as 39.0 µm. *In vitro* efficacy of the same was tested against *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Colletotrichum capsici*, *Rhizoctonia solani* and found effective even at 7 ppm concentration. Alghuthaymi *et al*. (2015) reported that most fungi have high tolerance towards metals and high wall binding capability, as well as intercellular metal uptake capabilities.

It is well known fact that there is a continuous interaction between fungus and the environment in which they live and it is the environmental condition that exerts an influence on growth and development and production of enzyme by the fungi. Therefore optimization of the role playing factors like temperature, pH, incubation period, biomass concentration and targeted type metal production affect the total quality and quantity synthesis of nanoparticle. Moreover the colloidal interaction conditions, that control size, shape, localization and dispersity of the nanoparticle formed also play an important role in synthesis of nanoparticle.

In the early 1990s, a thought process was triggered to employ such organisms which could biomineralise, for e.g. magnetotactic bacteria, which remove heavy metals and radionuclides from waste water, synthesise magnetic nanocrystals in magnetosomes, or bioremediate toxic metals by reduction of metal ions or formation of metal sulfides, or bioleach metals from ores, for e.g. gold from arsenopyrite ores, as possible nanofactories to produce nanomaterials. After bacteria and fungi many other types of organisms such as actinomycetes and algae were discovered which could help in nanomaterial synthesis (Ahmad *et al.* 2003). Some researchers even demonstrated synthesis of nanomaterials by some plants like alfalfa, capsicum, tamarind, *etc*. (Gardea *et al*. 2003). The nanoparticle synthesis mechanisms of unicellular and multicellular organisms may differ; however, the actual mechanisms are yet only laid down as hypothesis. The synthesis of nanoparticles by microbes is relatively a simple process which can be carried out with nutritional media and apparatus used regularly in a microbiology laboratory. In order to isolate microbes that are tolerant/ resistant to metals and can synthesise nanomaterials preferably soils from mining sites, sea-beds, *etc.* can be screened using the enrichment and isolation methods. Microbes produce nanomaterials either intracellularly i.e. in the cytoplasm, on the cell wall or in the periplasmic space, or extracellularly because of secretion of reducing and capping agents in the surrounding medium. Harvesting the intracellularly produced nanoparticles becomes a tedious process as the particles need to be separated from the organic matrix and the cell components whereas extracellularly produced nanoparticles are easier to harvest as they are free from the cell components. Therefore from the large scale production point of view extracellular nanoparticle production is less cumbersome hence more research has been done on this type of synthesis.

Changes in different parameters such as pH of the reaction mixture, temperature at which the reduction is carried out, concentration of the reducing agent, *etc*. lead to different shapes and sizes of the nanoparticles for e.g. nanospheres, nanotriangles, nanorods, *etc.* and different types of nanoparticles serve different purposes.

It has been reported by many researchers that use of fungi is highly potential for the synthesis as they produce and secrete large amounts of enzymes and they are easier to handle in the laboratory. Many bacteria have been shown to produce nanoparticles intracellularly for e.g. in the cell wall as in *Bacillus subtilis* or in the cytoplasm as in magnetotactic bacteria. A number of organisms can be employed for synthesising nanoparticles intra and extra-cellularly. which are presented in (Table 1).

Mechanism of synthesis of nanoparticle

Though much research has been attempted to find different methods to find out the possible mechanism but it is still an unsolved question. According to Sastry *et al.* (2003), fungal cell wall and cell wall sugar are likely to play an important role in the absorption and reduction of metal ions. The intercellular synthesis of nanoparticles can be explained using a stepwise mechanism. In the preliminary steps of bio-reduction, trapping of metal ions takes place at the fungal cell surface. This is probably due to the electrostatic interaction of the positively charged groups in enzymes present on the cell wall mycelia. In the next step, the metal ions are probably reduced by the enzymes within the cell wall, which leads to the aggregation of metal ions and formation of nanoparticle. Moreover, the extracellular synthesis of nanoparticle using fungi includes three mechanisms viz., nitrate reductase action, electron shuttle quinines or both.

Nanoparticles and control of phytopathogenic fungi

Some of the nano particles that have entered into the arena of controlling plant diseases are nano forms of carbon, silver, silica, ZnO and alumino-silicates.

Nanopesticides

Applications of nanotechnology within the agrochemical sector are only just emerging and many predict a rapid growth in coming years. In the past decade more than 3,000 patent applications have been lodged 60 peer-reviewed papers published, and 25 reports and reviews presented 50 dealing directly with nanopesticides, confirming the intensity of activity in this area. The term nanopesticidesis are used to describe any pesticide formulation that (a) intentionally includes entities in the nanometer size range (say up to 1000 nm), or is designated with a “nano” prefix (e.g., nanohybrid, nanocomposite), and/or or claimed to have novel properties associated with the small size. The aims of nanopesticide formulations are generally similar to those of other pesticide formulations, these being (a) to increase the apparent solubility of poorly soluble a.i. or (b) to release the a.i. in a slow/targeted manner and/ or protect the a.i. against premature degradation (Kah *et al*. 2013). Nanopesticides were first classified according to the intended purpose, with the objective of an analysing possible consequences affecting environmental fate.

Deliberate application of nanoparticles within agricultural practices could result in one of the rare intentionally diffuse inputs of engineered nanoparticles into the environment. The anticipated new or enhanced activity of nanopesticides will inevitably result in both new risks and new benefits to human and environmental health. It is unclear whether the current regulatory framework is adequate for the evaluation of these new products (Kah *et al.* 2013).

Applications of Nanotechnology in Agriculture

Nonmaterial's plays an important role in promoting sustainable agriculture and provide better foods globally (Gruère, 2012).In developing countries, nanotechnology has got important application for enhancing agricultural productivity, along with other emerging technologies such as pest management, biotechnology including genetics, plant breeding , disease control, fertilizer technology, precision agriculture, and other allied fields (Sastry *et al.* 2010). Nano encapsulation is process through which a chemical such as insecticides is slowly but efficiently released to particular host plant for insect pest control. Nanoscale systems like encapsulation and entrapment of agrochemicals such as fertilizers, pesticides, herbicides, plant growth regulators and other active substances by using polymers, dendrites, surface ionic attachments and other mechanisms may be used in controlled and slow release of agrochemicals, which allow the slow uptake of active ingredients and in turn of reduces the amount of agrochemical application by minimizing the input and waste. The nanoscale delivery vehicles increase effectiveness by binding firmly to the plant surface and reduces the amount of agrochemicals by preventing runoff into the environment (Chen and Yadav, 2011).

Nanotechnology can effectively use in pest management programme. Globally insect pests cause a huge crop loss of 14% and plant pathogens cause an estimated loss up to13% with a value of US $2,000 billion per year (Pimentel, 2009). Nano materials are use efficiently for safe administration of pesticides, herbicides, and fertilizers at lower doses. Pesticides cause adverse effects on human health and on pollinating insects. So, nanomaterials play an important role in decreasing toxicity and in turn help in increasing the efficacy of pesticides. Nano pesticide formulations increase the solubility of poorly soluble active ingredient and helps in releasing the active ingredient slowly. Lepidoptera is a second largest group of insects, with more than 150, 000 species worldwide, which causes yield loss up to 30-35% from sowing up to storage condition. There is hardly any cultivated plant that is not attacked by at least one lepidopteran pest.Caterpillars are major pests in forests, stored grains, and fibre and food crops. Fruit and shoot borer, Army worm, Rice moth and Diamond back moth were important Lepidoteran pests and which may destructive in field and storage conditions. They can be effectively controlled under field condition by applying green nanoparticles synthesized from plants like datura, tulasi and kaner. The scale of the result is great and this kind of research sprouts new platform for managing insect pests in eco friendly way.

Use of nano particles against fungal pathogen

Little is known about the effects of silver on phytopathogenic fungi because many studies have focused on bacterial and viral pathogens for animals. The antifungal activity of silver nanoparticles has a great potential for use in controlling spore-producing fungal plant pathogens. Nanometer-sized silvers possess different properties, which might come from morphological, structural and physiological changes (Nel *et al.* 2003).

It was also shown that the nano- particles efficiently penetrate into microbial cells, which implies that lower concentrations of nano-sized silvers would be sufficient for microbial control. This would be efficient, especially for some organisms that are less sensitive to antibiotics due to the poor penetration of some antibiotics into cells. A previous study observed that silver nanoparticles disrupt transport systems, including ion efflux (Morones *et al.* 2005). The dysfunction of ion efflux can cause rapid accumulation of silver ions, interrupting cellular processes at their lower concentrations such as metabolism and respiration by reacting with molecules. Also, silver ions are known to produce reactive oxygen species (ROS) via their reaction with oxygen, which are detrimental to cells, causing damage to proteins, lipids, and nucleic acids (Hwang *et al*. 2008). Most fungi have shown a high inhibition effect at a 100 ppm concentration of silver nanoparticles. In most cases, inhibition increases as the concentration of AgNPs is increased. It happens due to the high density at which the

Table. 1 : List of organisms synthesizing nanoparticles

Microorganism	Extracellular/ Intracellular nanoparticel synthesis	Metal
Actinomyctetes		
Rhodococcus	Intracellular	Au
Streptomyces sp.	Extracellular	Cu/ZnO
Algae		
Plectonemaboryanum	Intracellular	Au
Chlorella vulagaris	Extracellular	Ag
Bacteria		
Lactobacillus sp.	Intracellular	Au/Ag/Alloy of Au-Ag
Magnetospirillum magnetotacticum	Intracellular	Magnetite/Greigite
P. stutzeri AG259	Intracellular	Ag
Bacillus subtilis	Extracellular	Ag
B. licheniformis	Extracellular	Ag
Escherichia coli	Extracellular	Ag
Morganella sp.	Extracellular	Ag
Pseudomonas aeruginosa	Extracellular	Au
Rhodopseudomonas capsulata	Extracellular	Au
Rhodopseudomonas capsulata	Extracellular	Au
Stenotrophomonas maltophilia	Extracellular	Au
Fungi		
Aspergillus flavus	Intracellular	Ag
Rhizopus oryzae	Intracellular	Au
Trichothecium sp.	Intracellular	Au
Verticillium sp.	Intracellular	Au/Ag
A. fumigatus	Extracellular	Ag
Colletotrichum sp.	Extracellular	Au
F. solani	Extracellular	Ag
F. oxysporum	Extracellular	Magnetite/ Ag/ Au
Phanerochaete chrysporium	Extracellular	Ag
Trichoderma viride	Extracellular	Ag
Yeast		
Candida glabrata	Intracellular	CdS
Pichia jadinii	Intracellular	Au
Schizosaccharomyces pombe	Intracellular	CdS
Torulopsis sp.	Intracellular	PbS
Trichosporonovoides	Extracellular	Ag

solution was able to saturate and cohere to fungal hyphae and to deactivate plant pathogenic fungi. Upon treatment with Ag, DNA loses its ability to replicate, resulting in inactivated expression of ribosomal subunit proteins, as well as certain other cellular proteins and enzymes essential to ATP production. It has also been hypothesized that Ag^+ primarily affects the function of membrane-bound enzymes, such as those in the respiratory chain (Kim *et al.* 2012).

Two days after spray of nanosilver @ 10 ppm/ 500 kg effectively controlled rose powdery mildew, *Sphaerotheca pannosa* var. *rosae.* upto one week of its spray. Silver nanoparticle can strongly inhibited the the fungal growth and sclerotia

Table 2: List of botanicals used for synthesis of nanoparticles

Plant	Type of nanoparticles	Size	Reference
Aloe barbadensis	Au, Ag	50–350 nm	Chandran *et al.* (2006)
Azadirachta indica (neem)	Ag/Au bimetallic	50–100 nm	Shankar *et al* .,(2004)
Camellia sinensis	Ag, Au	30–40 nm	Vilchis-Nestor *et al,.*(2008)
Carica papaya	Ag	25–50 nm	Jain *et al.* (2009)
Catharanthus roseus	Ag	48–67 nm	Kannan *et al.*(2011)
Datura metel	Ag	16–40 nm	Kesharwani *et al.*(2009)
Ocimum sanctum	Ag	64.04 nm	Gogate *et al.* (2018)
Nerium olender	Ag	27.64	Gogate *et al.* (2018)
Paederia foetida	Ag	5-25	Bhuyan (*et al.*) 2017
Paederia foetida	Au	10-50	Bhuyan *et al.* (2017)
Nyctanthes arbor-tristris	Cu	11.58	Saikia *et al.* (2023)
Allamanda cathartica	Cu	4.05	Saikia *et al.* (2023)
Cascabela thevetia	Cu	16.96	Saikia *et al.* (2023)

germination of soil borne plant pathogen like *Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, S. minor* (Min *et al*. 2009). The microscopic data revealed that silver nanoparticle- treated hyphae were seriously damaged on hyphal walls, resulting in the plasmolysis of hyphae (Min *et al*. 2009). This study suggested the possible use of silver nanoparticles as an alternative to chemical pesticides for the eradication of phytopathogens even though there were some parameters to be evaluated for practical use. Jo *et al.* (2009) demonstrated antifungal activity of silver nanoparticles on phytopathogenic fungus *Bipolaris sorokiniana* (Sacc.) Shoem, which causes seedling blight, root rot, crown rot, and leaf spot blotch on various graminaceous species, and *Magnaporthe grisea* (Hebert) Barr, which causes blast on rice (*Oryza sativa* L.) and gray leaf spot on turfgrass. The efficacy of silver is greatly influenced by application timing, preventative applications of silver ions and NPs work better before spores penetrate and colonize within the plant tissue. Silver NPs (200 ppm) effectively reduced disease severity with an application at 3 h before spore inoculation, but their efficacy significantly diminished when applied

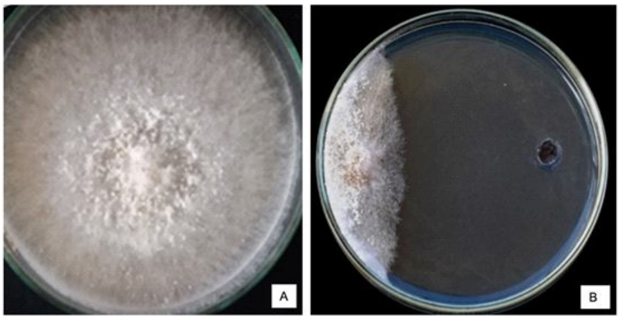

Fig.1: Effect of Silver nanoparticle (Ag NP) on *Rhizoctonia solani*. A- *R.solani* alone and B-Ag NP at 200 ppm + *R. solani*

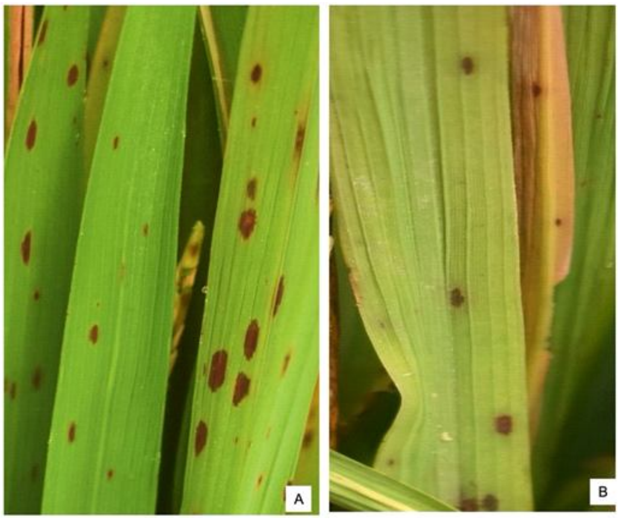

Fig 2 : *Bacillus subtilis* mediated green engineered ZnO particle loaded PGPR bioactive formulationon brown spot of rice in field condition. A- Controland B- Seed treatment + Foliar application @ 200 ppm)

at 24 h after inoculation. The *in-vitro* and in-planta evaluations of silver indicated that silver NPs influence colony formation of spores and disease progress of plant pathogenic fungi. In planta efficacy of silver NPs is much greater with preventative application, which may promote the direct contact of silver with spores and germ tubes, and inhibit their viability. Park *et al.*(2006) studied the effective concentration of nanosized silica-silver on suppression of growth of many fungi; and found that, *Pythium ultimum, Magnaporthe grisea, Colletotrichum gloeosporioides, Botrytis cinererea* and, *Rhizoctonia solani*, showed 100% growth inhibition at 10 ppm of the nanosized silica-silver. Whereas, *Bacillus subtilis, Azotobacter chroococum,Rhizobium tropici, Pseudomonas syringae* and *Xanthomonas compestris* pv.*vesicatoria* showed 100% growth inhibition at 100 ppm. They also reported chemical injuries caused by a higher concentration of nanosized silica-silver on cucumber and pansy plant, when they were sprayed with a high concentration of 3200 ppm.

Fungicidal activity of silver nanoparticle synthesized from *Trichoderma asperellum* at different concentration (100 ppm, 50 ppm, 30 ppm, and 10 ppm) was tested against four soil borne plant pathogens viz., *Rhizoctonia solani*, *Fusarium* spp., *Sclerotinia sclerotiorum*, and *Sclerotium rolfsii* and comparison was made with Carbendazim @ 3000 ppm. The result showed that the silver nanoparticles at 100 ppm significantly inhibit the mycelial growth of the pathogens as compared to Carbendazim (Kaman and Dutta, 2019). When the efficacy of silver nanoparticle as seed treatments was tested on growth parameters of four different crop plants at different concentrations, we found increased growth parameters (root and shoot length, fresh and dry weight of chilli, french bean, and mustard) as well as chlorophyll content up to 100 ppm concentration. Similarly, in a PhD Programme, silver nanoparticles synthesized from certain botanicals and when tested *in vitro*, it was found to be effective against lepidopteran pests of agricultural crops (Gogate *et al.* 2018 a & b). During a study on efficacy of biosynthesized silver nanoparticle from leaf of *Pderia foetida* (Vedailata in Assamese) showed inhibitory to human pathogen like *Staphylococcus aureus*, *Eschercia coli*, *Bacillus cereus* and *Aspergillus niger* (Bhuyan *et al*. 2017). In our study, silver nanoparticles synthesized from plant extract were found to increase the shelf life of cut flowers and also found to have antimicrobial activity during the storage. An experiment was conducted on silver nanoparticle to study the physiological effect on tea by different methods like leaf cutting, injection, spraying, and root dip treatment and found that foliar spray is found to be effective in increasing all the growth parameter as well as secondary metabolite like phenol, alkaloids, terpenoids etc (Ahmed and Dutta, 2019) . The same nanoparticles did not found to cause any harmful effect on the soil properties upto 100 ppm (Ahmed and Dutta, 2020).

Das and Dutta (2021) synthesized silver and gold nanoparticles through green approaches and were evaluated at 1, 5, 10, 50, 100 and 200 ppm for their antifungal activity against *Rhizoctonia solani* causing sheath blight of rice. Silver nanoparticle (Ag NP) at 200 ppm showed the highest inhibition (73.39%) in the radial growth of *R. solani*, (Fig. 1) while gold nanoparticle (Au NP) at the same concentration inhibited the growth of the pathogen up to 60.83%. Study on mode of action of nanoparticle by electron microscopy showed that Ag NP accumulate inside the fungal cells thereby cause distortion of fungal cells leading to death of the pathogen. Ag NP at 200 and 100 ppm caused complete inhibition of sclerotial germination of *R. solani*. Pot experiment conducted to study the efficacy of Ag NP at 200 ppm against sheath blight of rice showed that application of Ag NP increased the plant growth parameters as compared to control, with reduced per cent disease incidence (20.00%) as compared to inoculated control *R. solani* (88.00%). Application of Ag NP also increased the concentration of vital secondary metabolites like phenols, flavonoids, terpenoids and total soluble sugars.

Similarly ZnO nanoparticle synthesis from chemical process showed antimicrobial activity against fungi, bacteria and nematodes. Results showed that ZnO nanoparticle at a concentration of 225ppm showed highest antimicrobial activity against targeted organisms (Kaushik and Dutta, 2017). However when phytotoxicity was seen it was found that upto 100ppm ZnO nanoparticle does not showed any phytotoxicity effect but when increased to 225 ppm phytotoxicity was seen in the host. Cu nanoparticle synthesized from plant extract are found to have antifungal activity against the foliar diseases of Chrysanthemum. Recently Au nanopartilce synthesized from Cow urine, *Metarhizium anisopliae*, *Aspergillus niger*, black tulsi and bosch were found to have antifungal activity against the fungal diseases of rice.

As a nontoxic biodegradable material, as well as an elicitor, chitosan has the potential to become a new class of plant protectant, assisting towards the goal of sustainable agriculture. Addition of chitosan in *T. harzianum* medium has increased the antifungal activity against *F. oxysporum* and therefore could be considered as the alternative solution as biocontrol system of *Fusarium* wilt disease in tomato. In this direction, our preliminary data revealed that nanochitosan, when added to the growing media, enhanced the biocontrol potential of *T. asperellum* against soil borne plant pathogens including *Rhizoctonia solani* and *Sclerotium rolfsii* (applied as seed treatment). Chitosan nanoparticle synthesized by biological approaches proved to be have good antifungal activity against the diseases of vegetable crops (Boruah and Dutta, 2021). In the coming days we want to explore the scope of using *T. asperellum*-synthesized nanochitosan alone or in combination with its producer strain (*T. asperellum*) on the management fungal and bacterial plant pathogens of rice. Also we would like to analyse the transcripts of rice upon spraying with nanochitosan in comparison of the unsprayed control to understand the major transcripts associated with disease suppressive activity of the formulation.

In an experiment chitosan nanoparticle were isolated from four different sources viz., *Trichoderma viride*, *Metarhizium anisopliae*, *Beauveria bassiana,* and *Fusraium* spp. The isolated chitosan nanoparticle were characterized by using UV-Vis Spectroscopy, Dynamic Light Scattering (DLS) and Zetasizer. UV-Vis spectroscopic analysis and showed the yielding nanoparticle of size 5.615 nm to 329.0 nm. Chitosan nanoprtaicles when tested for its compatibility, it was found compatible with *T. asperallum* at 0.02 and 0.01 ppm. The compatible combination was further tested for its efficacy against three different pathogens such as *Fusarium* sp., *Sclerotium rolfsii,* and *Rhizoctonia solani* and comparison was made with Carbendazim @0.3 ppm. The result showed that the combined effect of Chitosan nanoparticle with *T. asperallum* was superior with 62.44% 47.55% and 44.88% mycelia growth inhibition of *Fusarium* sp., *S. rolfsii,* and *R. solani* respectively (Result of RKVY funded project on "Development of Next Gen Nanobioformulation of seed treatment of major crops") (Boruah and Dutta, 2021).

In a recent study it was found that *Bacillus subtilis* mediated green engineered ZnO particle loaded PGPR bioactive formulation when used as seed treating agent and foliar spray at 200 ppm significantly reduced the brown spot (Fig. 2) and bacterial blight of rice with highest plant growth parameter and major secondary metabolite like terpenoid, phenol, flavonoid (Unpublished data, 2022, applied for patent). The formulation showed compatibility with *Trichoderma harzianum, Beauveria bassiana, Metarhizium robertsii* and *Bacillus subtilis.* In an another study, a novel green engineered encapsulated silver-silica nanocomposite was developed and found effective for the management of sheath blight disease of rice withenhancement plant growth parameters and biochemical defense mechanism in rice and reduced per cent disease incidence of sheath blight disease. The product also helped the treated plants in nutrient uptake and also found compatible with biocontrol agents and a storage life of 120 days. The product can be a potential eco-friendly antimicrobial nanodrug in the near future (Unpublished data, applied for patent) as reported by Biswas and Dutta (2019).

CONCLUSION

Promising results and applications of nanoscience and nanotechnology are already being developed in the areas of delivery of pesticides, biopesticides, fertilizers and genetic material for plant transformation. By exploiting the unique properties of nanomaterials, the nanotechnologist has developed nanosensors which is capable for detecting pathogens at levels as low as ppb. Apart from detection, nanotechnology also has solutions for degrading persistent chemicals into harmless and sometimes useful components. Agricultural technology should take advantage of the powerful tools of nanotechnology for the benefit of mankind. The tools of nanotechnology can be employed to address the urgent issues of environmental remediation Development of a suitable nano-drug delivery method for ease of application and target specific action against phytopathogenic species along with constructing a suitable integrated disease management module to implement the nanodrug in combination with cultural, physical and biological practices can be a potential area of further study.

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DECLARATION

Conflict of Interest. Authors declare no conflict of interest.

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