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Review Article

Agronanotechnology for Plant Fungal Disease Management: A Review

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A B S T R A C T

After the introduction of fungicides in 1940, there has been negligible impact on the living system of this planet. The fungicides used for disease control irrationally posed danger to living system killing not only the target fungi but also affecting beneficial living systems. To preserve biodiversity, it is becoming necessary to reassess our strategies and achieve disease management by alternate approaches such as nanotechnology. Biocontrol agents such as Phytocompounds and biopesticides are still projected as alternatives to the synthetic fungicides. Nanofungicides based on silver nanoemulsion tackle fungal pathogen problems in agriculture and hold promise in the future. World over scientists have been propagating this branch of science through their research, but very little is done to bring this technology at farmers level.

Keywords

Nanotechnology, Silver nanoemulsion, biocontrol, nanofungicide

Introduction

Crop cultivators continually battle with fungal diseases affecting their crops. Plant pathogenic fungi must be controlled if consumer demand in developed countries for premium quality and diverse foods is to be met; while high quality cereals, fruits and vegetables are an indicator of economic growth in developing countries. Commercial agriculture relies heavily upon high inputs of chemical pesticides to protect crops against pathogens and pests. The resistance of pests and plant pathogens against pesticides and fungicides is rapidly becoming a serious problem.

These practices are currently being re-evaluated due to concerns about the possible health and environmental consequences. Growing consumer awareness towards the use of these chemical compounds is beginning to direct farmers into the investigation of alternative chemical control measures. Nanotechnology has emerged as a tool to explore the darkest avenues of Science and technology research and promises a pool of research and opportunities in areas of medicine and healthcare, biotechnology, materials and manufacturing, energy, information technology in conjunction with the national security. It is strongly expected that this technology would bring next industrial revolution.

Nanotechnology refers to a nanoscale technology, which has promising applications in day-to-day life. This technology emphasizes the implications of individual atoms or molecules or submicron dimensions in terms of their applications to physical, chemical, and biological systems and eventually their integration into larger complex systems. The word nanotechnology is generally used when referring to materials with the size of 0.1 to 100 nanometres (Morones *et al.,* 2005).

Nanotechnology tenders prospects to re-explore the biological properties of already known antimicrobial compounds by manipulating their size to alter the effect. 'Nanobiotechnology' is a new branch of biology that has originated due to the compatibility of nanosized inorganic and organic particles with biological functions. Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals or ceramics, which can display several biological applications. Different types of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver have been developed, but silver nanoparticles (Nano-Ag) have proved to be most effective as they exhibit potent antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms (Guo *et al.*, 2003). Nowadays, nanosilver is found in clothing, food containers, wound dressings, ointments, implant coatings, and other items; some nanomaterial applications for use in food packaging and food processing material have received approval from the US Food and Drug Administration. The "First Green Revolution" promised food to everyone, but over the years agricultural production is now experiencing a plateau, which is compelling the need for "Second Green Revolution" to meet the expanding population's food demand (Singh, 2012). Nanoscale science and nanotechnologies are envisioned to have the potential to revolutionize agriculture and food systems (Norman and Hongda, 2013) and has given birth to the new era of "Agronanotechnology". The antimicrobial properties of silver have been documented since 1000 B.C., when silver vessels were used to preserve water, but its applications in the field of agriculture have gained momentum very recently. 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.

In this review, we have addressed various problems associated with the existing methods of fungal phytopathogen control and also the emphasis has been laid upon the use of AgNPs as Ag nanoemulsion to control phytopathogens in the field and application of AgNPs along with the biofertilizers to supplement their effect and promote the plant growth in a better way.

Plant parasitic fungi and crop loss

Fungi are responsible for more than 70% of all major crop diseases (Agrios, 2005) and significant crop loss is observed in several crop species like rice, wheat, barley, cotton, groundnut and grapevine (Dhekney *et al.*, 2007). In agriculture, annual crop losses due to pre- and post harvest fungal diseases exceed 200 billion euros, and, in the United Stated alone, over $600 million are annually spent on fungicides (Fernandez *et al.,* 2010). Nearly one quarter of food crops worldwide are affected by mycotoxins such as aflatoxins, ergot toxins, *Fusarium* toxins, patulin and tenuazonic acid (Schneider and Ullrich, 1994).

Although the majority of fungal species are saprophytes, a number of them are parasites, with around 15,000 of them, causing disease in plants, the majority belonging to the Ascomycetes and Basidiomycetes (Grover and Gowthaman, 2003) (Table 1). According to the type of infection strategy and parasitism, fungi have been classified as necrotophic (e.g., *Botrytis cinerea*),

biotrophic (e.g., *Blumeria graminis*) or hemibiotrophic (e.g., *Colletotrichum destructivum*). While the former derives nutrients from dead cells, the latter takes nutrients from the plant but does not kill it. Only a limited number of fungal species successfully infiltrate and invade host tissues, thus they bypass host recognition and plant defense responses to obtain nutrients from them. Eventually they may develop disease in plants and sometimes host death. The life cycle of fungal pathogens is complicated involving with both asexual and sexual reproduction. Further, the disease cycle involves establishment of infection, colonization, growth, reproduction, dissemination of the pathogen and survival of the pathogen in the absence of the host. However, each stage is accomplished in different manner depending on the pathogen (Fernandez *et al.,* 2010).

For designing efficient crop protection strategies, knowledge of the pathogenic cycle and virulence factors is needed. These details can be helpful in the development of resistant plant genotypes through either classical plant breeding or genetic engineering. Further, these strategies are complemented by the application of chemical fungicides or the use of biological control as well. Plenty of research has been performed on plant-fungal pathogen interactions using numerous approaches, ranging from classical genetics, biochemistry and cell biology to the modern, holistic and high-throughput omic techniques followed by bioinformatic tools. The study of fungal plant pathogens has expanded in the past decade due to the availability of their genomic sequence data and resources for functional genomics analysis, including transcriptomics, proteomics, and metabolomics. These studies in combination with targeted mutagenesis or transgenic studies have revealed host-pathogen communication at the molecular level and all the complex mechanisms involving pathogenesis and host avoidance (Walter *et al.,* 2010).

Fungal plant diseases are generally managed with the applications of chemical fungicides. Chemical control has been found very effective for some fungal diseases, but it leaves several non-specific effects that destroy beneficial organisms along with pathogens. Such ecological disturbances open the route to undesirable health, safety, and several environmental risks (Manczinger *et al.,* 2002). A promising method for protecting plants against diseases is constructing and employing pathogen-resistant cultivars. Although a number of resistant cultivars have been developed through breeding programs, these cultivars become obsolete in a short time due to the rapid evolution of the phytopathogens and the emergence of virulent forms capable to overcome the plant resistance. Breeders are often confronted with the issue of using a limited number of plants in their breeding programs, undesirable traits transferred together with the valuable resistance genes, and, in recent years, also with the depletion of potential gene sources.

Control of Phytopathogenic fungi

Physical methods

Soil solarization' is one of the physical methods that are based on disinfestations of soil by the heat generated from trapped solar energy. Trapping solar energy and elevating the soil temperature to control soil pests is an age old practice. Soil solarization process controls the soil pathogenic organisms efficiently by trapping solar energy under cold frames subjected to direct sunlight (before planting) for sufficient periods so as to raise the temperature of the top layer of soil (to a depth of 10 cm) to 40°-60°C. The black root rot of tobacco seedlings caused by *Thielaviopsis basicola* were controlled by employing such treatments. Incidence of disease occurrence caused by *Sclerotium rolfsii* and the effects of solarization on this pathogen has been described in various studies. Sclerotial viability of *S. rolfsii* was quickly reduced by more than 95% at 2.5 cm depth in solarized fruit orchards soil, though lowering effects were found in deeper soil layers (Shlevin *et al.,* 2003).

Limitations of Physical method

Soil solarization is more selective towards thermophilic and thermotolerant microbiota, actinomycetes may survive and even flourish under soil solarization (Gamliel *et al.* 1989). The major constraints that limit the adoption of soil solarization in practice are relatively longer duration of the process and the climatic dependency. The cost of solarization is relatively low compared with other available alternative; however, it can be a limiting factor depending on the country, the crop type, the production system (e.g. organic versus conventional farming) and the cost and availability of alternatives. Application of soil solarization as a non-chemical tool for weed management was proven to be more cost-effective and profitable than methyl bromide or some other treatments (Boz *et al.,* 2004), especially in high-income crops (Vizantinopoulos and Katranis, 1993). The general limitations of soil solarization is the requirement of crop-free fields for relatively longer periods (1-2 months) during summer season; survival of pathogens at deeper layers in the soil; possible pollution from plastic residues after the treatment termination; lack of adequate machinery for large scale soil, mulching in developing countries; some pests are not controlled or difficult to control by this treatment; high wind and animals may tear the plastic.

Chemical methods

Plant pathogens are estimated to cause yield reductions of almost 20% in the principal food and cash crops worldwide. Although these losses may be minimized by the use of disease tolerant cultivars, crop rotation or sanitation practices, but fungicides and pesticides are often essential to maximize crop yields. Dutta and Das, (2002) studied *in vitro* efficacy of thiram and mancozeb at 0.1 per cent concentration against tomato isolate of *S. rolfsii* and reported that thiram had inhibited 70.3 percent of mycelial growth and 96.5 per cent of sclerotial production in *S. rolfsii*. Bhat and Srivastava, (2003) evaluated *in vitro* efficacy of captan, thiophanate-methyl and propiconazole at 250, 500 and 1000 ppm concentrations against *S. rolfsii*. They found that propiconazole was effective even at 250 ppm concentration against *S. rolfsii*.

Bhuiyan *et al.* (2012) carried out several studies on the effect of chemical fungicides in inhibition of the *S. rolfsii* mycelial growth. The investigation used different fungicides like Ridomil, Rovral, Tilt, Dithane, Bavistin, and Provex at various concentrations. At 400 ppm concentration, inhibition of the mycelial growth was 52.9%, 93.88%, 100%, 80.63%, 6.64% and 100% respectively. The study revealed that provex inhibited radial mycelial growth totally even at low concentration of 100ppm.

Fungicide resistance and other limitations of chemical method

Fungicide resistance (lack of sensitivity) is a major cause of poor disease control in fungal pathogens. The development of fungicide resistance is influenced by complex interactions of factors such as the mode of action of the fungicide (how the active ingredient inhibits the fungus), the biology of the pathogen, fungicide use pattern, and the cropping system. Understanding the biology of fungicide resistance, how it develops, and how it can be managed is crucial for ensuring sustainable disease control with fungicides.

The problem of fungicide resistance became apparent following the registration and widespread use of the systemic fungicide benomyl (Benlate) in the early 1970s. Resistance problems appeared a few years after benomyl was introduced where the fungicide was used intensively. Fungicide resistance is now a widespread problem in global agriculture. Fungicide resistance problems in the field have been documented for more than 100 diseases (crop - pathogen combinations), and within about half of the known fungicide groups. Zaki *et al.* (1998) have described that the effectiveness of synthetic fungicides suffers from several drawbacks such as potential harmful effects on non target organism and environment, development of resistant races of pathogens, and possible carcinogenicity of some chemicals.

Biological methods

Fungal plant diseases are usually managed with the applications of chemical fungicides. For some diseases, chemical control is very effective, but it is often non-specific in its effects, killing beneficial organisms as well as pathogens, and it may have undesirable health, safety, and environmental risks (Manczinger *et al.,* 2002).

Classical methods: A promising method for protecting plants against diseases is constructing and employing pathogen-resistant cultivars. *Brassica* crops are heavily challenged by various fungal pathogens and insects, whereas bacterial and viral diseases have little effect on their yield (Abdel-Farida *et al*., 2009). The different available control measures that are considered effective against the pathogens have been investigated and breeding practices are considered most effective for brassica (Srivastava *et al.,* 2011). The evolution of phytopathogens is rapid and their emerging virulent forms can therefore effectively circumvent the plant resistance, thus making the resistant varieties ineffective.

Biocontrol agents: The potential for the use of fungal antagonists as bio-control agents of plant diseases were suggested by Weindling (1932), who was the first to report the parasitic activity of *Trichoderma* spp. against *Rhizoctonia solani* and *S. rolfsii*. Parikh and Jha, (2012) demonstrated the biocontrol potential of bacterial isolate LK11 against *S. rolfsii* during in planta studies.

Dutta and Das, (2002) studied the antagonistic potential of *T. harzianum, T. viride* and T. *koningii* against tomato isolate of *S. rolfsii* by dual culture technique. Several microbes serving as antagonists like *Trichoderma* sp., *Gliocladium virens* and *Pseudomonas fluorescens* from rhizosphere soil of sunflower infected with *S. rolfsii* have also been reported. Bhuiyan *et al.* (2012) reported that *T. harzianum* isolates collected from rhizosphere and rhizoplane of different crops were screened against *S. rolfsii* following dual plate culture technique. Dube (2001) described the mechanisms of disease suppression by rhizobacteria. He stated that rhizobacteria antagonize soil borne pathogens through the production of antibiotics or lytic enzymes (chitinase) and through competition for nutrients, notably iron as well as by inducing systemic resistance in the plant against subsequent infection by pathogens.

Biotechnological tools: Our knowledge of molecular events occurring during plant–pathogen interactions have expanded significantly in the last ten years. Based on this knowledge, several strategies have emerged for developing crop varieties resistant to pathogens. The most significant development in the area of varietal development of disease resistance is the use of the techniques of gene isolation and genetic transformation to develop transgenic resistance to fungal diseases. Genetic engineering technology has proved to be beneficial in managing viral (Wani and Sanghera, 2010) and bacterial (Sanghera *et al.,* 2009) diseases in plants. Biotechnology has enhanced our understanding of the mechanisms that control the plant's ability to recognize and defend itself against disease caused by fungi (Punja, 2007). The integration of biotechnology with traditional agricultural practices will be the backbone for sustainable agriculture.

Limitations of biological method

The practical efficiency of transgenic plants with disease resistance genes in the field is merely in coherence with the *in vitro* results. Thus, the need for field productivity of any biological and biotechnological approach should be addressed. Interestingly, the selection of resistant cultivars through breeding practices has been found most suitable for the control of phytopathogenic fungi for application of fungicides has been prohibited due to their environmental impact and concern.

Controlling *S. rolfsii* disease using *Trichoderma* in the field is difficult, because the pathogen has a wide host range and can survive in the soil for a long time. It takes more intensive management and planning such as proper education and training to achieve success through biological control. Further, an understanding of the biology of pests and their enemies is essential for successful application of biological control. Additionally, biological control practices are sometimes costlier and dramatic results are also not seen as compared with chemical fertilizers.

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 and alumino-silicates.

Nano Carbon

Carbon's uniqueness in several ways has helped mother nature to select it as the brick molecule for simple as well as complex architectural designs of almost all molecules. At such a situation Nanotechnology has astonished scientific community, because at Nano-level material shows different properties. Thus we are exposed to a huge spectrum of Nanosciences, wherein there are totally new materials, new technologies and new hope for existing problems related to agrochemicals, pesticides, herbicides regulation and smart utilization. Brazilian agriculture research corporation's areas of focus are to include research for producing carbon nano-fibers to strengthen natural fibers for example, those from coconuts and sisal and making nanoparticles that contain pesticides and control their release. Scientists are mostly concentrating on carbon nanotubes (CNT). Carbon nanotubes are allotropes of carbon, whose nanostructure is cylindrical in shape. Recently, scientists (Khodakovsky *et al.*, 2000) reported that when they planted tomato seeds in a soil that contained carbon nanotubes; these CNTs could not only penetrate into the hard coat of germinating tomato seeds but also exerted a growth enhancing effect. They envisaged that the enhanced growth was due to increased water uptake caused by penetration of the CNT. This could be a boon for using CNT as a vehicle to deliver desired molecules into the seeds during germination that can protect them from the diseases. Since it is growth promoting, it will not have any toxic or inhibiting or adverse effect on the plant.

Nano Alumino-Silicate

Leading chemical companies are now formulating efficient pesticides at nano scale. One of such effort is the use of aluminum-Silicate nanotubes with active ingredients. The advantage is that Alumino-Silicate nanotubes sprayed on plant surfaces are easily picked up in insect hairs. Insects actively groom and consume pesticide-filled nanotubes. They are biologically more active and relatively more environmentally-safe pesticides.

Mesoporous Silica Nanoparticles

Wang *et al.* (2002) have shown that mesoporous Silica nano particles can deliver DNA and chemicals into plants, thus, creating a powerful new tool for targeted delivery into plant cells.

Lin's research group has developed porous, silica nanoparticles systems that are spherical in shape and the particles have arrays of independent porous channels. The channels form a honeycomb-like structure that can be filled with chemicals or molecules. These nanoparticles have a unique "capping" strategy that seals the chemical inside.

Nano-emulsions

It is a mixture of two or more liquids (such as oil and water) that do not easily combine. In nano-emulsion, the diameters of the dispersed droplets are 500 nm or less (Figure 1). Nano-emulsions can encapsulate functional ingredients within their droplets, which can facilitate a reduction in chemical degradation (McClements and Decker, 2000).

Nano Silver

Nano silver is the most studied and utilized nano particle for Bio-system. It has long been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. Silver nanoparticles, which have a high surface area and high fraction of surface atoms, have high antimicrobial effect as compared to the bulk silver. Nano silver colloid is a well dispersed and stabilized silver nano particle solution and is more adhesive on bacteria and fungus, hence are better fungicide. As mentioned earlier Silver is known as a powerful disinfecting agent. It kills unicellular microorganisms by inactivating enzymes having metabolic functions in the microorganisms by oligodynamic action. Silver in an ionic state exhibits high antimicrobial activity (Thomas and McCubin, 2003). However, ionic silver is unstable due to its high reactivity and thus gets easily oxidized or reduced into a metal depending on the surrounding media and it does not continuously exert antimicrobial activity.

Preparation methods of silver nano emulsion

Silver nano emulsion contains nanoparticles of silver between 1-100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. There are many different synthetic routes to silver nanoparticles. They can be divided into three broad categories: physical vapor deposition, ion implantation, or wet chemistry. Among these, wet chemistry is most widely used method that employs preaparation of silver nanoparticles by simple coreduction of silver metal ions using sodium citrate in aqueous medium.

Wet chemistry method

The advantage of chemical synthesis is its versatility in designing and synthesizing new materials that can be refined into the final product. The primary advantage that chemical processes offer over other methods lies in good chemical homogeneity, as chemical synthesis offers mixing at the molecular level. Molecular chemistry can be designed to prepare new materials by understanding how material is assembled on an atomic and molecular level and the consequent effects on the desired material macroscopic properties.

Guzman *et al.*, 2009 described the method of preparation of Silver nanoparticles by the chemical reduction method. Silver nitrate was taken as the metal precursor and hydrazine hydrate as a reducing agent and Sodium Dodecyl Sulfate (SDS) or citrate of sodium were stabilizing agent. The formation of the silver nanoparticles was monitored using UV-Vis absorption spectroscopy. The UV-Vis spectroscopy revealed the formation of silver nanopartícles by exhibiting the typical surface plasmon absorption maxima at 418-420 nm from the UV–Vis spectrum.

Ion implantation method

Popok *et al.*, 2005 investigated the process of metal nanoparticle (NP) synthesis in SiO_2 by implanting silver ions with an energy of 30 keV and the ionic current density (4–15 $\mu A/cm^2$). Analysis of the composite materials formed was performed with the use of optical spectroscopy and atomic-force microscopy (AFM).

Physical vapor deposition

A direct, synthetic method for preparing narrowly dispersed silver nanoparticles without using size-selection processes were described by Lin *et al.* (2003) by thermal reduction of silver trifluoroacetate in isoamyl ether in the presence of oleic acid. This direct synthesis is versatile, easy to control, synthetically, and able to make Ag nanoparticles with diameters in the range of 7−11 nm with narrow size distribution. The diameter of these nanoparticles can be tuned by varying the oleic acid to silver trifluoroacetate molar ratio. The particles were characterized by transmission electron microscopy, selected area electron diffraction, ultraviolet-visible spectroscopy, and powder X-ray diffraction.

Silver nano particles as effective antifungal agents

Silver nanoparticles are the most studied and employed nano particles for biological system. These nanoparticles have been known for their strong bactericidal and inhibitory effects in addition to a broad spectrum of antimicrobial activities. The studies on the applicability of nano-silver for controlling plant diseases has been limited till date (Lamsal *et al.,* 2011). Silver nanoparticles, which have a high surface area and high fraction of surface atoms, have high antimicrobial effect as compared to the bulk silver.

Rose powdery mildew caused by the fungi *Sphaerotheca pannosa Var rosae* is a very widespread and common disease of both greenhouse and outdoor grown roses. It causes leaf distortion, leaf curling, early defoliation and reduced flowering. Double capsulized nano silver was prepared by chemical reaction of silver ion with the aid of physical methods, reducing agent and stabilizers. They were highly stable and very well dispersive in aqueous solution. The nano silver colloidal solution of concentration of 5000 ppm was diluted in 10 ppm of 500 kg and sprayed at large area of 3306 m^2 polluted by rose powdery mildew. Two days after the spray, more than 95% of rose powdery mildew faded out and did not recur for a week.

Several members of plant pathogenic fungi belonging to ascomycetes and basidiomycetes develop sclerotia, including *Sclerotinia sclerotiorum, Sarracenia minor, and Rhizoctonia solani.* Sclerotium forming pathogens are widespread in the world and cause many important diseases in a wide host range of plants. As primary surviving structures of the pathogens, sclerotia exhibit longevity in soil and resistance to unfavorable abiotic factors such as heat, drought, and fungicide (Coley-Smith, 1979). Diverse disease management, such as chemical methods and genetic controls has been used for the control of the diseases caused by sclerotium-forming fungi. However, their broad host range and the formation of sclerotia make it difficult to control diseases. 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. The field study has also suggested that, the efficacy of silver is greatly influenced by application time and preventive applications of silver nanoparticles work better before fungal isolates penetrate and colonize within the plant tissue (Lamsal *et al.*, 2011).

The antifungal activity of silver nanoparticles was evaluated against sclerotium-forming phytopathogens, *R. solani*, *S. sclerotiorum*, and *S. minor* during a study conducted by Min *et al.* (2009). The data demonstrated that the nanoparticles strongly inhibited the fungal growth and sclerotial germination growth. It is also suggested that 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 solution was able to saturate and cohere to fungal hyphe 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).

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). His 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. These may involve the evaluation of phytotoxicity and antimicrobial effects in hosts, and development of delivery systems of silver nanoparticles into host tissues colonized by phytopathogens. Therefore, these unanswered questions were addressed in the investigation performed in our laboratory and the fungicidal effects of the silver nanoemulsion during '*in planta*' studies were evaluated. It was found that the growth of *Sclerotium rolfsii* was strongly inhibited and in turn the silver nanoemulsion promoted the growth of mung bean plants. Similar studies have been reported for the activity of silver ions and nanoparticles against the devastating phytopathogens; *Bipolaris sorokiniana* and *Magnaportha grisea* (Agrawal and Rathore, 2014). Toxic effects of silver nanoemulsion were observed at higher concentrations in our study. Thus, before recommending the use of emulsion in agricultural fields, the toxic levels should be tested and an optimum concentration should be advised.

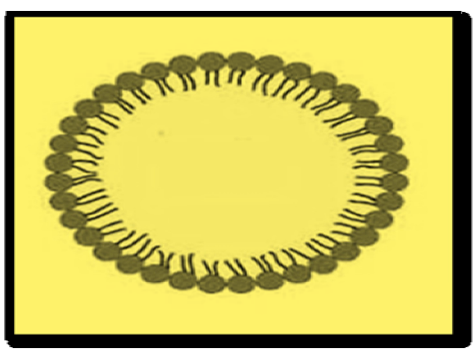
Silver is now an accepted agrochemical replacement. It eliminates unwanted microorganisms in plants, soils and hydroponics systems. It is being used as a foliar spray to stop fungi, moulds, rot and several other plant diseases. Moreover, silver is an excellent plant-growth stimulator. Silver in the form of a metal or oxide, is stable in the environment. Nano-silica, silver prepared by exposing a solution including silver salt, silicate and water soluble polymer to radioactive rays, showed antifungal activity and controlled powdery mildews of pumpkin at 0.3 ppm in both field and greenhouse tests. Park *et al.* (2006) also 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 have 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.

No wonder that maximum patents are filed for 'Nano silver for preservation and treatment of diseases in agriculture field.' This popularity of nano silver has caused concern about regulating and classifying the nano silver as a pesticide (Anderson, 2009). In May 2008, the International Center for Technology Assessment (ICTA) submitted a petition to EPA requesting that it regulate nano-silver used in products as a pesticide under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Table.1 Yield loss in some major crops of India mediated through fungal diseases

Crop	Pathogen	Disease	Total yield loss (%)
Rice	*Pyricularia oryze*	Blast	21
Wheat	*Puccinia recondiata*	Leaf rust (Brown rust)	30
Maize	*Helminthosporium maydis* and *H. turcicum*	Leaf blight	30
Sorghum	*Sphacelotheca reiliaria*	Grain mould	18
Pigeonpea	*Fusarium udum*	Wilt	24
Chickpea	*Fusarium oxysporum*	Wilt	23
Brassica	*Alternaria brassicaceae*	Blight	30
Soybean	*Phakospora packyrhizi*	Rust	23
Potato	*Phytophthora infestans*	Late blight	31

Figure.1 Schematic representation of nanoemulsion



Fungal diseases are responsible for significant failures in crop yield. However, understanding the epidemiological conditions can help in the control of disease. Above review provides a comprehensive assessment of the different practices being used for the control of the plant fungal disease however, breeding techniques seem to be the best approach among existing methods to improve the genetic line of plants as the field efficiency of the genetically engineered plants is questionable. Besides, several natural biological processes take place in the nanometre scale regime; thus, a confluence of nanotechnology and biology can address several agricultural problems such as controlling phytopathogens, and can update the available biocontrol measures in the field of agriculture. It is presumed that the use of silver nanoemulsion will augment the biofertilizer application towards enhanced agricultural productivity.

In summary, the application of silver nanoparticles would open a vista of research in integrated manner such as pathogen control, plant-pathogen interactions, integrated pest management and many more. However, several aspects of silver nanoparticles with relation to plants viz., its half-life in soil, its toxicity effects on plants and the optimum dosage for application in the field needs to be determined. There are some questions still remaining to be addressed, such as the exact mechanism of interaction of silver nanoparticles with fungal cells and how the surface area of nanoparticles influences killing activity.

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