Chapter 4
Myco-nanotechnology in Agriculture

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4.1 Introduction

A fungus is a eukaryotic organism that obtains its food by absorbing nutrients from the host through its cell wall. The branch of biology deals with fungus is known as mycology; mycology is originated from a Greek word μύκης *mykes* means mushroom. Fungi are extensively studied by Antonio de Michelli who is recognized as father of modern mycology. Fungal body (thallus) is composed of tubular microscopic thread like hyphae and reproduces by the formation of spores. Fungi differ from plants as they possess chitin in their cell wall instead of cellulose (Duran et al. 2005). Fungi are heterotrophic organisms which means they rely on other organisms or materials for their nutrition or food. They have not evolved the photosynthesis as a biological activity. They get nutrition by absorbing nutrients from their host (Parasites) or from dead organic material (decomposers) by secreting digestive enzymes extracellularly. Fungi are entitled to be the main decomposers of natural ecosystems. There is a great diversity in types of fungi and the habitat they live in. There is an estimation of 1,500,000 species of fungi on globe, but very few are identified. Now with the modern research tools, there is a hope that we may be successful to get more fungal strains in near future, with an approximation of nearly five million (Musarrat et al. 2010).

Fungi are considered as quite ordinary due to their smaller sizes and mysterious life cycles. Although they are having very important roles in ecosystems like they are parasites of plants and animals, symbiotic partners of algae and plants. Therefore they are having main and vital roles in recycling of nutrients between biotic and abiotic components of ecosystems (Heinrich and Wojewoda 1976). Some ecologically important and common types of fungi are given in Fig. 4.1.

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Fig. 4.1 Some ecologically significant fungi (**a**) *Penicillium notatum* (**b**) *Lentinula edodes* (**c**) *Agaricus bisporus* (**d**) *Amanita muscaria*

Fungi reproduce asexually as well as sexually; sometimes a unique condition of reproduction also prevails in kingdom fungi known as parasexuallity. A fungus in which both asexual and sexual mode of reproduction are present is known as a Holomorphic fungus. If only asexual stage is present than fungus is said to be Anamorph while if only sexual stage is present than the fungus is a Telomorphic fungus.

There are different methods of asexual reproduction prevailing in the kingdom fungi. Most common of which are by spores, conidia and mycelial fragmentation. Spores are vegetative cells or bodies produced inside the sporangium while conidia are vegetative bodies cut off from the tips of certain hyphae known as conidiophores. Mycelial fragmentation is the phenomenon of breakdown of mycelia into small fragments and growth of each fragment into a new organism. Sexual reproduction gives two main benefits; one is the rapid and continuous dispersal of population and secondly to maintain the population adapted to a specific niche. Common modes of sexual reproduction in kingdom fungi are isogamy, anisogamy and oogamy. However, there is one class of fungi with no or unknown sexual stage in their life cycle and are known as imperfect fungi or Deuteromycota (Castro-Longoria et al. 2011). Fungi may have haploid, diploid or heterokaryon stage in their life cycle. In sexual life cycle of reproducing fungi, hyphae fuse to form a diploid hyphae or hyphae with heterokaryons (nuclei from the two parents don't fuse but stay together). This process is often referred as anastomosis and it is the first step for the sexual stage of the life cycle of such fungi (Shankar et al. 2003). Fungi are identified and grouped on the basis of morphology of fruiting bodies/sexual parts. For example members of Ascomycota and Basidiomycota have asci and basidia respectively as fruiting bodies containing ascospores and basidiospores.

4.2 Fungi and Agronomy

Fungi have both positive and negative impacts on agronomy. In one aspect fungi are notorious parasite and pathogen of large number of economically important crops. On the other hand fungi may act as biofertilizers in the form of mycorrhizal association with about 99% of the plant families. There are a number of pathogenic fungi causing different diseases in different plants. These diseases are mainly recognized and diagnosed by the type of host plant, plant part affected and the pattern/type of symptoms on infected part. A large number of fungi are parasitic and pathogenic in nature, as they use living host for their food, this makes them detrimental for crop plants (Kumamoto and Vinces 2005). Fungi parasitize various economically important plants including rice, wheat, potato, tomato, sugarcane, cotton. On basis of above facts, some of the general types of fungal diseases are discussed below

- Anthracnose
- Damping-off diseases
- Downy mildews
- Grey mold
- Leaf spots and blights
- Powdery mildews
- Root and foot rots
- Rusts
- Smuts

4.2.1 Damping-Off of Seedlings

Damping-off is a fungal disease in which fungus attack on growing seedlings. Damping-off is produced by various species of the fungal genera like *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium*. These all fungal strains are facultative parasites and cause almost same symptoms, so categorized as causative agents of damping off. The term "damping-off" is used because causative agents of this disease are mostly active in damp soils. As *Phytophthora* and *Pythium* species give rise to zoospores that need water for their movements in soil pores. Damping-off fungi are part of soil microbiota; they compete for organic material with other microbes present in the rhizosphere of the plants. If there is no organic material or host available for these fungi, they can form resistant resting structures like oospores in *Phytophthora* and *Pythium*, chlamydospores in *Fursarium solani* and sclerotia in *Rhizoctonia solani*.

This soil borne disease is categorized as important fungal disease causing much loss to the growing crops. It is characterized into two types

(a) Pre-emergent damping off which includes the decaying of seedlings and finally collapsing before they emerge from soil.
(b) Post emergent damping off which is the rotting and downfall of seedlings at soil level.

Seedlings of most plant species are susceptible to damping-off. This disease also attack on the fleshy and storage organs of important commercial plants. But when plants survived at the seedling stage, they are then not killed by damping-off fungi (Liao et al. 2000). Spores of damping off fungi (zoospores, oospores, mycelial hyphae) produce germ tubes and penetrate into seedlings tissues where they grow inter-cellularly as well as intra-cellularly (Kalo-Klein and Witkin 1990). They cause the breakdown of host tissue by secreting digestive enzymes and causing death to the plants.

4.2.2 Rot and Foot Rots

Root systems of a number of economically important crops are infected by "Rot and Foot Rots". These rots are caused by fungi and cause rotting of the roots and lower or basal portion of the stems adjacent to the roots. Due to the rotten roots, these plants can't absorb sufficient water and nutrients to maintain normal growth. As a result, stem and leaves are stunted and yellow; and they finally wilt and die. Sometimes damage due to infection is compensated or hidden by the growth of new roots (Volesky and Holan 1995).

There are four causative fungal groups for root rots. First group are the fungi with restricted host range and live as saprophytes, example includes *Gaeumannomyces graminis*. Second are the fungi which are saprophytic in nature and have a range of host crops that form resting spores e.g., *Rhizoctonia solani*. Third group includes *Sclerotinia sclerotiorum*, *Scterotium rolsfii*, which survive by producing sclerotia. Last group is host restricted but they do not form resistant bodies like *Fusarium* spp. such as *Fusarium oxysporum* and *Fusarium solani*.

4.2.3 Take-All of Wheat

It is also root disease found in wheat crop and caused by a fungus known as *Gaeumannomyces graminis* var. tritici. It is reported to be very common in areas where winters receive good rainfall and these soils are lighter, poorly drained, nutrient deficient with basic pH. This fungus may be restricted to a few plants in a field or may infect a larger area in patches.

Daniel McAlpine in 1902 described that this infection is also called "whiteheads" because of the destruction in root structure, growth of wheat plants is stunted and premature ripening occurs. Due to this earlier ripening, spikes appear as white heads among green normal spikes. These white head spikes are usually empty or with very small wrinkled grains. This may cause a serious damage to the crop and large reductions in the yield (Kumamoto and Vinces 2005). The take-all fungus survives in summer by infecting other herbs or grasses nearby in fields or by living saprophytically in the debris of wheat crop because it does not make resistant resting spores. This survival time period is highly influenced by the physical and chemical status of the soil environment as well as the local weather. It can survive for maximum of 2 years without host, therefore it is relatively easy to control.

4.2.4 Downy Mildews

The downy mildew fungi are obligate parasites and have a wide range of hosts. They cause severe losses to grains, vines, ornamentals and fruits. A number of fungal classes cause this infection. The name of disease is given due to the fact that primary infections are apparent on the surface of leaves, branches and fruits of infected plants in form of 'downy bloom' mainly containing sporangiophores and sporangia. Some of the common downy mildew causing fungi are *Plasmo parahalstedii*, *Sclerospora philippinsts*, *Plasmopora viticola* and *Perono sclerospora maydis*. These fungi cause systematic infections like at first chlorosis in form of streaks on leaves and then production of sporangia later on. Downy mildews can cause extensive damage to crops in years when the environment favors infection. A loss of US$250 million was estimated in USA and Canada due to the attack of downy mildew on tobacco crop (Liao et al. 2000).

4.2.5 Leaf Spot and Blight Diseases

This is common group of disease caused by a number of fungi and it's a very common infection in plant species, ranging from crops of agronomic importance to wild plants of natural communities. These infections can be seen on leaves and on other above ground parts of plants. The leaf spot causing fungi are usually belonging to the order Dothidiales of the Ascomycota group of fungi.

One of the famous example is Ascochyta blight of chickpea which caused a complete loss of crop a number of times. Disease is identified by the circular lesions on pods and foliage, elongated lesions on stem and petioles. It showed that whole aerial parts of plants come under attack of the pathogen. Circular rings like lesions on leaves and pods in fact have pycnidia, which produce two celled microspores in wet season. Therefore they come out in large numbers and attack tendrils of other plants. This disease prevails in areas of world with humid and cold climate and diseased seeds and crop leftovers are the main surviving spaces for the fungus between seasons (Mukherjee et al. 2001a).

4.2.6 Grey Mold Disease

Botrytis cinerea Pers. Ex Fr. is the causative agent of gray mold, one of the most destructive plant pathogen known to man. It causes both pre and post-harvest infections in a number of plants like strawberry, tomato, grapevine, chick pea, bulb flowers, cucumber, potato, onion and other ornamental plants. This disease is a serious concern in countries particularly where environment favors the growth of mold like Australia, Argentina, Pakistan, Nepal, India and Bangladesh (Shahiduzzaman 2015).

4.2.7 Rusts

Rusts are another diverse group, having some unique characteristics, causing havoc to crops of commercial importance. Causative agents belong to the order Uredinales of Basidiomycota and can infect and survive on living host only. Almost 168 rust genera and approximately 7000 species have been reported out of which half belongs to the genus Puccinia.

But luckily, steps can be taken to lower the losses caused by these pathogens as they are very much host specific but don’t cause the infection in non-host plants. A hurdle in research is the resistance in growth in pure cultures by these fungi, which causes delays in the research process at lab level. Rust fungi complete their life cycles in two separate hosts with different types of spores. These different types of spores are host specific in fact and cannot grow on the other host. Infections of rust fungi are marked by the rust like fruiting bodies having spores on the surface of leaves, petioles, stem, tender shoots, fruits etc. These rust spots may be of different colors like yellow, orange, black etc. Rust infection causes the stunted appearance of the infected plants with yellow leaves (Latif et al. 2018).

4.2.8 Smuts

The word "Smut" is derived from the German word which means smoke or dirt. This name was given to the infection because the inflorescence infected by smut releases the fungal spores in the form of smoke or dust. Smuts are pathogens of cereal crops infecting the members of Poacea and Cyperaceae. Important host plants are wheat, maize, rye, grass, oat, sugarcane and other grasses. Smuts belonging to the class Ustilaginales of class Basiodiomycota, mainly attack on leaves and stems by developing sori within the plant tissue. Finally they hijack the inflorescence and reproductive parts of the plant where they form galls full of thick walled black teliospores which when released gives smoky appearance and hence get the name (Wunderle et al. 2012).

4.2.9 Anthracnose

Anthracnose is caused by *Colletotrichum gloeosporioides* and characterized as appearance of dark lesions. It has been reported to cause yield losses (34–47%) to fruit plants especially mango. This disease affects a number of plants but most affected crop is of mango. It infects the flower sets of mango trees and caused the losses in fruit production in warm and humid conditions of climate (Sundravadana et al. 2007; Pandey et al. 2012). *C. gloeosporioides* secretes various enzymes like Polygalacturonase, Polygalacturonase transeliminase and cellulase that might be responsible for its pathogenicity (Jat et al. 2017).

In conventional agricultural practices, fungi are mainly controlled by use of different chemicals which are also called fungicides. But there is an increasing public concern about the overuse and misuse of fungicides. These fungicides on one side control the fungal diseases but on the other hand environmental pollution is cumulative day by day due to over use of fungicide. Increased research in the area of fungal role in agronomy can make a milestone in modern agronomic techniques. An alternative of fungicide is the use of natural compounds or biological control. In nature many plant and plant families possess antifungal constituents in the form of secondary metabolites. Use of nanotechnology and nanoparticles is another solution to avoid fungicide consumption to protect environment.

4.3 Role of Fungi in Nanoparticles Synthesis

There are large numbers of living organisms, which give a chance to biotechnologists to explore and exploit them for the wellbeing of human race. One of them is to synthesize nanoparticles by using fungal organisms directly or by using their metabolites and extracts. Huge amounts of fungal enzymes which take part in NPs

Table 4.1 List of fungi that synthesize metal nanoparticles

Sr. #	Type of nanoparticles	Fungal species	References
1	Ag (silver nanoparticles)	*Alternaria alternata*	Kareem et al. (2019), Sarkar et al. (2011)
		Alternaria solani	Amal and Ghazwani (2015)
		Aspergillus flavus	Ninganagouda et al. (2013)
		Aspergillus niger	Gaikwad and Bhosale (2012)
		Fusarium oxyporum	Amal and Ghazwani (2015)
2	Au (gold nanoparticles)	*Alternaria alternata*	Dhanasekar et al. (2015), Sarkar et al. (2012)
		Aspergillus sidowii	Vala (2015)
		Rhizopus oryzae	Das et al. (2009)
		Thermophillic fungi	Molnar et al. (2018)
		Rhodococcus sp	Ahmad et al. (2003)
3	Cu (copper nanoparticles)	*Neurospora crassa*	Rashmi et al. (2004)
		Stereum hirsutum	Cuevas et al. (2015)
		Ureolytic fungi	Li and Gadd (2017)
4	Fe (iron nanoparticles)	*Alternaria alternata*	Mohamed et al. (2015)
		Aspergillus oryzae	Raliya (2013)
		Verticillium sp.	Bharde et al. (2006)
5	Si (silica nanoparticles)	fungus	Bansal et al. (2005)
6	Zn (zinc nanoparticles)	*Aspergillus fumigatus*	Velmurugan et al. (2010) Baskar et al. (2013)

formation can be obtained on commercial level in fermenters (Pimprikar et al. 2009). Myconanotechnology is a new field of research making its roots in last decade. To synthesize metal nanoparticles with success, a substantial number of fungal species are widely used like *Fusarium oxysporum*, *Rhizopus oryzae* and *Verticillium* sp. (Table 4.1). The utilization of biomass of fungi and/or cell free extract for the synthesis of metal NPs yielded in different shapes and sizes of these myconanoparticles (Narayanan and Sakthivel 2010). Both spherical and quasi-spherical silver NPs were produced from *Fusarium oxysporum* with size varying from 20 to 50 nm (Bharde et al. 2006). Extract of *Rhizopus oryzae* was used in manufacturing of the diverse shape gold nanoparticles and it depends on gold particle concentration, pH value and reaction time (Binupriya et al. 2010).

4.3.1 Mechanisms Behind Myconanoparticles Synthesis

Several promising mechanisms have been recommended for the development of metal nanoparticles, but no such mechanism has been known yet and extensive research is still needed. According to Mukherjee et al. (2001a, b) mainly the cell wall and sugar component of the fungal cell wall involve in the process of bio-reduction of the metallic ions. Nanoparticles are formed on the exterior of fungal cell wall, and the very basic step is bio-reduction to trap the metallic ions. The electrostatic interactions between charged group on the cell wall surface and metal ions followed by metal ions enzymatic reduction lead to the accumulation and formation of nanoparticles.

Birla et al. in 2009 suggested that fungal cell wall proteins also play a substantial role in the formation of zirconia nanoparticles. Fungi secrete hydrolyzing protein in acidic condition that makes binding with zirconium to form the zirconium NPs. These NPs forming proteins are cationic in nature with a molecular weight of 55 kDa. *Verticillium* sp. also produces these cationic proteins which might be the cause of hydrolysis of ferric ions (Bharde et al. 2006). According to Ahmad et al. (2003) tryptophan and tyrosine are the amino acids that play a pivotal role in the bio reduction of metal ions to metallic nanoparticles. NADH-dependent enzymes and the fungal proteins are also involved in the metal ion reduction (Germain et al. 2003).

4.3.2 Fungi a Renewable Source for Nanoparticles Synthesis

Fungi are fascinating source for the green synthesis of nanoparticles owed to their metal bioaccumulation capacity. Furthermore, fungi are easy to grow in the laboratory and production of large quantity of biomass make them valuable to be used in the green synthesis of NPs (Kumar et al. 2011). Fungal cell wall possess a number of functional groups that make bonds with the metal ions and this high wall-metal binding capacity makes fungi more attractive than other microbes (Maynard and Michelson 2006). Biosynthesized nanoparticles are environment friendly and are biocompatible for pharmacological uses. Biosynthesis of nanoparticles by fungi as a base material may be a reasonable approach. Fungal enzymes possess high redox potential which makes them more suitable for the oxidation reduction reaction for the conversion of metallic ions into specific nanoparticles. So, the green synthesis of nanoparticles is now an attractive field around the globe. Figure 4.2, Illustrates Advantages of fungi as bio-factories for nanoparticles synthesis.

4.4 Myconanoparticles Application in Management of Fungal Diseases

Myconanotechnology is a rising field, in which fungi can control the synthesis of nanostructures with required size and form. Mycosynthesis of various types of nanoparticles is carried out using metal salts such as silver, gold, titanium, selenium, platinum, palladium, zirconia, tellurium, cadmium, telluride, silica and magnetite gold-silver alloy. Currently, silver nanoparticles have attracted the scientists due to their extensive application in the areas like biomedicine, agriculture, physics and microbial biotechnology as antimicrobial agents at the nano-scale (Kowshik et al. 2002).

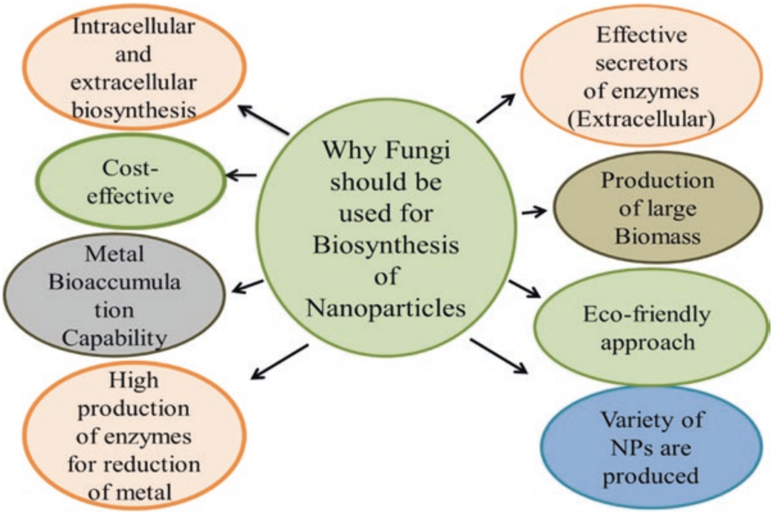

Fig. 4.2 Advantages of fungi as bio-factories for nanoparticles synthesis

Phytopathologists are working in search of techniques to protect economically important crops from destructive plant pathogens. Nanotechnology provides as alternative way for the production of pesticides and fertilizers which are safer to environment. Sanghi and Verma (2010) suggested that nanoparticles are found effective against pests, nematodes and fungal plant pathogens.

Though, fungus based green synthesis of myconanoparticles and their extensive range of applications (Fig. 4.3) have fascinated the attention of investigators. Several noticeable applications of nanotechnology in different areas of agronomy have been described in subsequent sections:

4.4.1 Nanoparticles as a Suppresser for Pests (Nanopesticides)

The use of AgNPs as antimicrobial agents has been exploited in controlling plant diseases. Nanoparticles have potentially shown various modes of inhibitory action against plant pathogens. The use of biosynthesized AgNPs by exploiting biological agents for the effective management of plant disease has attained significant consideration now a day. The reason of this approach is the low cost and less toxicity of nanoparticles to biomolecules. Furthermore, biosynthesized AgNPs are equally capable to those nanoparticles produced by chemical methods. Nanoparticles offer non-toxic environment friendly method for the management of plant diseases against phytopathogens (Feng et al. 2000).

Plants are directly or indirectly influenced by various types of biotic stress such as disease and as well as abiotic stress such as drought, salinity, heat, flood, etc.

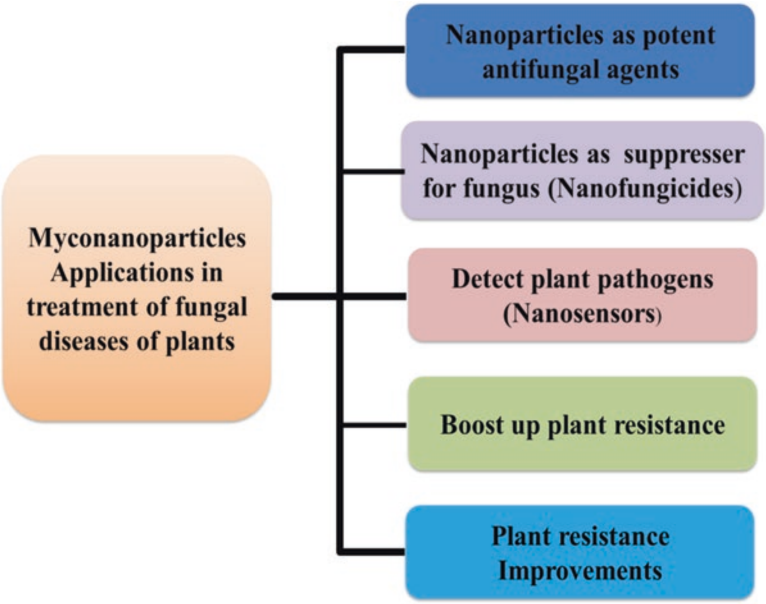

Fig. 4.3 Potential Myconanoparticles applications in plant pathology

which is responsible for destructive economic loss. Nowadays, to avoid such type of damages, breeders developed resistant varieties of plants against them. The Nano-biotechnology improves plant resistance by implying novel measures using NPs like, nano capsules and nanofibers, to manipulate genes to increase plant resistance (Mukherjee et al. 2002).

It is possible to use nanoparticles against various types of plant pathogens because it is safer in comparison to synthetic fungicides, for example-Ag-SiO_2 NPs have significant antifungal activity against *Botrytis cinerea* (Riddin et al. 2006). Germain et al. (2003) also reported that combined application of a fungicide such as fluconazole and Ag NPs, can effectively suppress the growth of *Phomaglomerata*, *Phomaherbarum*, *Candida albicans Trichoderma* sp. and *Fusarium semitectum*. Soil borne plant pathogens produce hard structures in the form of sclerotia which are difficult to manage. These sclerotia are basically survival structures contain melanin to overcome environmental stress and are resistant to chemical and physical degradation.

Sclerotium rolfsii is a devastating soil borne plant pathogen which causes collar rot in chickpea and leads to about 50–95% mortality at seedling stage (Gericke and Pinches 2006). Sun and Xia (2002) suggested that there is a direct interface between biosynthesized AgNPs and sclerotia. The scleortia of *S. rolsfii* show reduced germination by the application of AgNPs as these AgNPs produce very responsive Ag^+ ions which then show their antimicrobial activity to stop the growth of fungus. Unquestionably the diffusion into the microbial cell, disturbance in transport system, accretion of Ag^+ ions and fabrication of reactive oxygen species are the certain approaches of movements of AgNPs responsible for its antimicrobial property (Sarkar et al. 2012).

In last few years, the silver based nanopesticides are rapidly developed by the researcher for the management of plant pathogens due to their antimicrobial property which is proved after application against various plant pathogens; however, it is safe or nontoxic to humans. The larger surface-to-volume ratios of AgNPs elevate their interaction with microbes and their capacity to infuse (Velmurugan et al. 2010). AgNPs based nanopesticides cause inhibition in the hyphal growth of *Rhizoctonia solani*, *Sclerotium sclerotiorum* and *S. minor* (Thakkar et al. 2010).

Similarly Das et al. (2010) reported that biosynthesized silver nanoparticles using *Stenotrophomoas* sp. can be used for the management of soil-borne and foliar phytopathogens. A mixture of AgNPs with amphiphilic hyper branched macromolecules can be utilized as an effective antifungal surface coating (Ahmad et al. 2006). Antifungal activity of silver nanoparticles against 18 plant pathogens was also described by Castro-Longoria et al. (2011).

Silica is known to have prominent antifungal potential as it increases the hydrophobic pressure of leaves which may trigger the plant defense towards biotic and abiotic stresses. Nano-silica increases the defense response towards fungal infection in maize over bulk silica treatment (Verma et al. 2010). This is possibly because of the mechanism of increased silica transport and deposition in roots as well as in leaves. Thus, it is evident that nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the cell wall can easily penetrate and reach plasma membrane. There is an enlargement of pores or induction of new cell wall pores on interaction with nanoparticles which in turn enhances the uptake of water and nutrients of plants (Tian et al. 2010). In addition, deposition of amorphous silica in the cell walls leads to leaf erectness and hence, may prevent the invasion of pathogenic fungi. Thus the nanosilica can be used for active defense mechanism to improve crop protection.

4.4.2 Myconanoparticles Mechanism of Action Against Fungal Pathogens

The exact mechanism of growth arrest and destruction of fungal pathogen of plants by myconanoparticles is not well understood. Microorganisms are believed to use some enzymes to metabolize oxygen to sustain life. Silver ion particles scrapple the enzyme and stop oxygen metabolization. This suffocates the fungi and other microorganism, leading to death (Longoria et al. 2012). Currently, fungus based AgNPs are being completely surveyed and extensively investigated as potential antifungal agents. Their tiny size and high surface-to-volume ratio boost their interaction with fungal species to carry a diverse range of antifungal activities (Blackwell 2011). The suppressive effect of AgNPs on fungi has led to follow many mechanisms. It is assumed that AgNPs possess high affinity towards sulphur and phosphorus in the cell. As silver ions (Ag^+) from AgNPs move with DNA containing phosphorous element moieties, this leads to inactivation of DNA replication. Interaction of AgNPs with proteins containing sulphur which is present within or outside the cell is considered to be another reason. AgNPs become connected to sulphur-containing proteins of microorganism cell membranes resulting in high permeability of cell membrane, leading to the death of microorganisms (Ahmad et al. 2002). Studies on the dose dependent effects of AgNPs (in the range of 10–15 nm) on microorganisms showed that at a micromolar level of these Ag^+ ions can inhibit enzymes participating in respiration or may interfere in permeability of membrane to protons and phosphate. At higher concentrations these ions can move within nucleic acids and cytoplasmatic elements (Das et al. 2009). As fungi contain large quantities of protein product, they're most popular as new economical sources for nanostructure formulation (Alvarez-Puebla et al. 2004).

4.5 Future Prospective

The production of metallic nanoparticles by fungi appears to be a relatively simple biotechnological process, predominantly involving only the reaction of fungal culture filtrates with solutions of metal salts. There are, however, a number of issues where further research is required. The work undertaken to date indicates that there may be a number of different kinds of reducing agents involved in the mechanism of synthesis of metal nanoparticles. These may also have effects on the final shapes and size of the nanoparticles. There is also a need to search out the specific mechanisms which involved in nanoparticle formation from fungi, and for comparative studies to determine whether the same or different pathways are used by different fungi for different metals. Once the basic mechanisms have been determined there will be a need to evolve mechanism for optimizing the specific concentrations, sizes and shapes of the nanoparticles. For the synthesis of commercially feasible myconanaoparticles, it would be necessary to develop low-cost recovery techniques to isolate the nanoparticles from the fungal mycelium for the easy use of these NPs in industrial processes. More researches need to be carried out on applications of myco-nanoparticles for plant disease treatment by a targeting particular disease and their controlled release.

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