Chapter 4
Myconanotechnology in Agriculture

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Abstract Nanotechnology is a fast-growing field of science that involves synthesis and development of various nanomaterials, production, manipulation and use of materials ranging in size from less than a micron to that of individual atoms. Formation of nanoparticles employing fungi and their application in medicine, agriculture and other areas is known as myconanotechnology. Fungal nanoparticles could be used in various fields including agriculture, industry and medicine. In the present chapter, the status of research carried out on fungal nanoparticles in the area of agriculture is consolidated and presented. Myconanotechnology has emerged as one of the key eco-friendly technologies, and its use in management of bacterial and fungal diseases, pest control, preserved foods and beverages is constantly being explored. Thus, myconanotechnology provides a greener alternative to chemically synthesized nanoparticles. Mycosynthesized nanoparticles found their vast application in pathogen detection and control, wound healing, food preservation, textile fabrics and many more. The present chapter provides an appraisal on the application of myconanotechnology in agriculture and looks into the future prospects.

4.1 Introduction

There is a rapid growth in the field of nanotechnology which involves the synthesis and production of various nanomaterials and nanostructures. The synthesis of nanoparticles with fungi is known as myconanotechnology. Fungi produce large amount of enzymes which can be used for the synthesis of nanoparticles. There are three methods in the synthesis of metal nanoparticles such as physical, chemical and biological methods. Among these, biological synthesis is favoured than chemical and physical synthesis due to their fast synthesis and ability to control shape and

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size. Therefore, they are of cost-effective and environment friendly in nature (Saxena et al. 2014). Different fungi have been investigated for the synthesis of nanoparticles such as gold, silver, selenium, platinum, zinc oxide, titanium nanoparticles, etc. by various researchers. Fungi have numerous advantages from other organisms in the synthesis of nanoparticles. They are easy to isolate and handle and are capable of secreting extracellular enzymes (Mandal et al. 2006; Mohanpuria et al. 2007; Alghuthaymi et al. 2015) and have the ability to withstand flow pressure than bacteria and plants (Narayanan and Sakthivel 2010). In addition, the process of synthesis has a greener approach as it is nontoxic and occurs at low cost. Fungi-based synthesis of nanoparticles has received much attention to researchers due to their extensive advantages in different fields. Fungal nanoparticles can be used in various fields like agriculture, engineering, pharmaceuticals, environment, textiles, medicine, food industry, etc. (Dasgupta et al. 2015; Nanda and Majeed 2014; Yadav et al. 2015; Prasad et al. 2016). Thus myconanotechnology provides a greener alternative to chemically synthesized nanoparticles. The present chapter provides the application of myconanotechnology in agriculture and its future perspectives.

4.2 Synthesis of Fungal Nanoparticles

Fungal nanoparticles can be synthesized both intracellularly and extracellularly (Fig. 4.1). There have been several reports on the intracellular and extracellular synthesis of nanoparticles using fungi. The work done by researchers in the synthesis of fungal nanoparticles is listed in (Table 4.1). Nanoparticles are fabricated inside the cell of the fungus in intracellular where the biomass of the fungus is reacted with a metal, whereas in extracellular synthesis, the filtrate of the fungus reacts with the solution of a metal (Yadav et al. 2015). Electrostatic interactions occur during intracellular synthesis where ions of the metal bind upon the fungal cell. The ions of the metal are reduced by the enzymes present in the cell wall, and then formation of nanoparticles occurs due to aggregation of the metal ions. During extracellular synthesis, the fungus, when exposed to the metal ions, leads to release of reductase enzymes and formation of nanoparticles, which are highly stable (Kashyap et al. 2013). A rapid extracellular and intracellular biosynthesis of gold nanoparticles using the fungus *Penicillium* sp. was reported by Du et al. (2011). Intracellular synthesis of gold nanoparticles was obtained when $AuCl_4^-$ ions reacted with the cell filtrate of the fungus in 1 min, whereas extracellular synthesis occurred when solution of $AuCl_4^-$ incubated with fungal biomass for 8 h. There are two different methods for the preparation of extracellular biosynthesis, i.e. rapid synthesis and slow synthesis, whereas intracellular biosynthesis is a time-limiting factor which depends on in vivo synthesis of cells (Moghaddam 2010). Due to an additional step required to obtain the purified nanoparticles, the extracellular synthesis method is more favourable than intracellular method (Kuber et al. 2006).

The extracellular and intracellular syntheses of fungal nanoparticles reported by various researchers are described below.

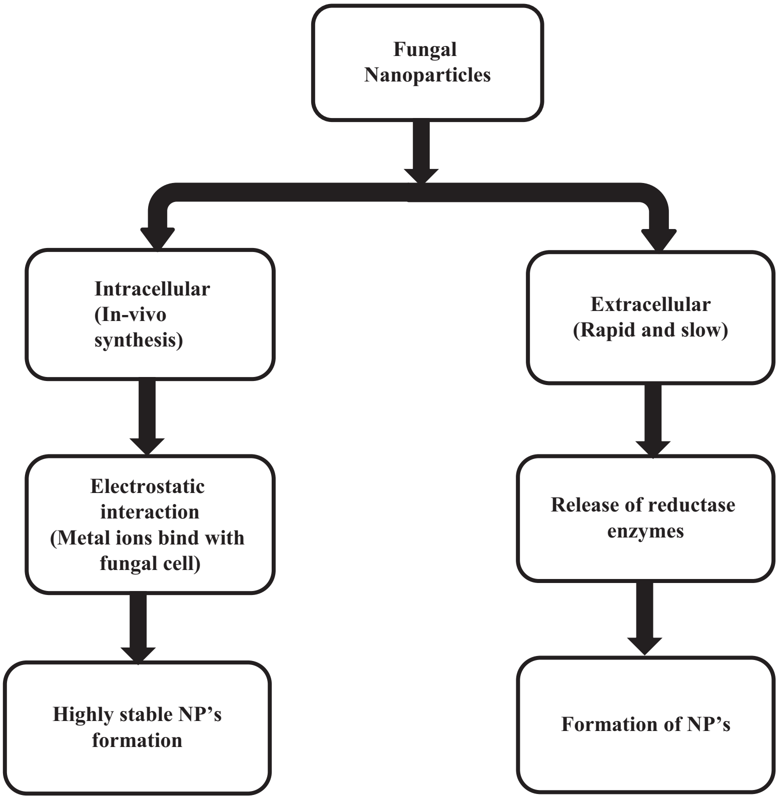

Fig. 4.1 Mycosynthesis of nanoparticles

Fatima et al. (2016) reported the mycosynthesis of silver nanoparticles using *Aspergillus flavus*. The synthesized nanoparticle was found to be spherical in shape with 50 nm in size which showed antimicrobial effect against pathogenic fungi and bacteria. Further, it is also reported to be a microbicidal agent in the field of agriculture. In another study conducted by Ingle et al. (2009), *Fusarium solani* was reported to be a new biological agent in the extracellular synthesis of silver nanoparticles. Fourier transform infrared spectroscopy (FTIR) revealed the silver nanoparticle to be highly stable due to the presence of capping agent. Extracellular synthesis of gold nanoparticles using *Fusarium oxysporum* f. sp. *cubense* JTI and its antimicrobial activity against *Pseudomonas* sp. was reported by Thakker et al. (2013). The mycelium reacted with auric chloride and changed the yellow colour to purple colour within 60 min indicating the formation of gold nanoparticles with particle size of 22 nm. The extracellular synthesis of gold nanoparticles using *Helminthosporium tetramera* was studied by Shelar and Chavan (2014). The synthesized gold nanoparticle was found to be polydispersed spherical with size range of 8–50 nm. This study would be appropriate for establishing a process for large-scale manufacturing of scanty AuNPs. Zinc oxide nanoparticles against two

Table 4.1 List of mycosynthesis of nanoparticles

Fungal species	Nanoparticle	Synthesis	Size (nm)	Reference
Aspergillus flavus (KF934407)	Silver	Extracellular	50	Fatima et al. (2016)
Fusarium solani	Silver	Extracellular	5–35	Ingle et al. (2009)
Fusarium oxysporum f. sp. *cubense* JT1	Gold	Extracellular	22	Thakker et al. (2013)
Helminthosporium tetramera	Gold	Extracellular	8–50	Shelar and Chavan (2014)
Botrytis cinerea and *Penicillium expansum*	Zinc oxide	Extracellular	70	He et al. (2011)
Aspergillus terreus	Zinc oxide	Extracellular	54.8–82.6	Baskar et al. (2013)
Coriolus versicolor	Silver	Intracellular and extracellular	10	Sanghi and Verma (2009a, b)
Saccharomyces cerevisiae	CdS	Extracellular	2.5–5.5	Prasad and Jha (2010)
Coriolus versicolor	CdS	Extracellular	10	Sanghi and Verma (2009a, b)
Aspergillus flavus	Titanium dioxide	Extracellular	62–74	Rajakumara et al. (2012)
Alternaria alternata	Selenium	Extracellular	13–15	Sarkar et al. (2011)
Fusarium oxysporum	Platinum	Extracellular	5–30	Syed and Ahmad (2012)
Mucor hiemalis	Silver		5–15	Aziz et al. (2016)

pathogenic fungi, i.e. *Botrytis cinerea* and *Penicillium expansum*, and its antifungal activity were investigated by He et al. (2011). The results suggest that zinc oxide nanoparticles could be used in agriculture as a productive fungicide and applications in food safety. Baskar and his co-workers also studied a greener synthesis of zinc oxide nanoparticles against *Aspergillus terreus*. The synthesized crystalline zinc nanoparticles were characterized by UV absorption spectrum, X-ray diffraction spectrum and Fourier transform infrared spectroscopy and were found to be spherical in shape, and scanning electron microscope revealed the size range from 54.8 to 82.6 nm. The synthesized zinc oxide nanoparticle was found to be a potent antifungal agent against fungal species (Baskar et al. 2013).

An intracellular synthesis and extracellular synthesis of silver nanoparticles using the fungus *C. versicolor* was demonstrated by Sanghi and Verma (2009a) reported for the first time. The fungus when treated with silver nitrate solution agglomerated the silver nanoparticles on its surface in 72 h. The reaction was quick and could easily continue at room temperature even without stirring under alkaline conditions. The resulting AgNPs displayed controllable structural and optical properties depending on the experimental parameters such as pH and reaction temperatures. The average size, morphology and structure of particles were characterized by

TEM, AFM, XRD and UV-vis spectrophotometry. The FTIR study imparts that the particles were bound by the amino groups which was responsible for the nanoparticle stability. Another study of intracellular and extracellular synthesis of gold nanoparticles using an alkalotolerant fungus *Trichothecium* sp. was investigated by Ahmad et al. (2005). Gold ions when reacted under stationary conditions, with the fungal biomass, produced extracellular synthesis, while the biomass reaction with agitating conditions resulted in intracellular growth of the nanoparticles. The synthesized gold nanoparticles were found to be spherical and triangular in morphology. They demonstrated that altering the conditions of the reactions of the fungal biomass and gold ions resulted in the intracellular and extracellular synthesis, where under stationary conditions, the enzymes and proteins are released into the medium but are not released under shaking conditions.

A rapid and eco-friendly method of biosynthesis of CdS nanoparticle using *S. cerevisiae* was reported by Prasad and Jha (2010). Another extracellular synthesis of CdS nanoparticle was studied by Sanghi and Verma (2009b) using an immobilized fungus *Coriolus versicolor.* The first report on fungus-based biosynthesis of titanium dioxide nanoparticle was investigated by Rajakumara et al. (2012). The synthesized nanoparticle was found to have good antibacterial property. Scanning electron microscopy disclosed the particle to be oval and spherical shapes with size being in the range of 62–74 nm. Sarkar et al. (2011) reported for the first time in synthesizing selenium nanoparticles with *Alternaria alternata.* The synthesized nanoparticles were characterized by atomic force microscopy, dynamic light scattering and transmission electron microscopy and revealed the nanoparticle size to be in the range of 30–15 nm. An extracellular synthesis of platinum nanoparticles using *Fusarium oxysporum* was studied by Syed and Ahmad (2012). *Fusarium oxysporum* reacted with hexachloroplatinic acid resulted in the formation of selenium nanoparticle with size in the range of 5–30 nm which are highly stable. Because of their high stability, ability not to flocculate and having a good monodispersity, they may find applications in various fields.

4.3 Factors That Affect Nanoparticles Mycosynthesis

There are several factors that affect the synthesis of fungal nanoparticles such as temperature, biomass, concentration and time in exposure of substrate, pH and the presence of particular enzyme (Fig. 4.2). These are known to be the major factors that affect the shape and size of nanoparticles (Kashyap et al. 2013). Khan et al. (2016) studied the optimization of various parameters such as pH, quantity of fungal biomass, temperature and concentration of silver nitrate in the synthesis of silver nanoparticles from *Aspergillus niger*. They concluded that optimizing the above parameters will enhance the silver nanoparticles synthesis as well as its yield. Optimizing the cultural and physical conditions in the synthesis of silver nanoparticles from *Fusarium oxysporum* has also been studied by Birla et al. (2013).

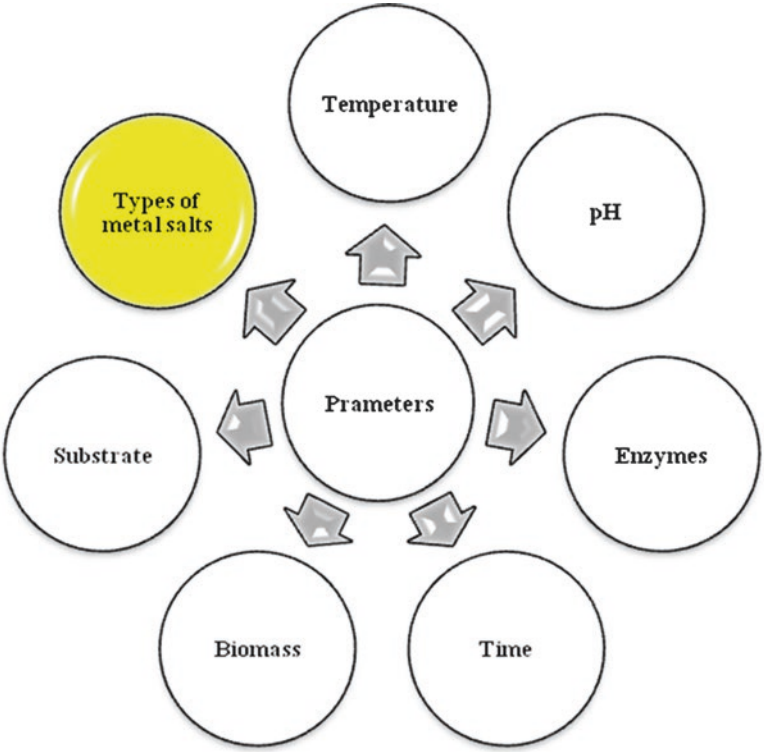

Fig. 4.2 Parameters effecting mycosynthesis of nanoparticles

One of the most important factors affecting the mycosynthesis of nanoparticles is pH. It greatly influences the nature and size of the nanoparticles synthesized (Gardea-Torresdey et al. 1999; Armendariz et al. 2004). The synthesis of gold nanoparticles with *Aspergillus terreus* and its antibacterial property against *Escherichia coli* was studied by Priyadarshini et al. (2014). The nanoparticle was found to be 10–19 nm at pH 10 based on UV-vis spectroscopy, XRD, TEM, EDX and FTIR results which revealed the characteristic property of the synthesized nanoparticle. Temperature plays an important role in regulating the activity of the fungus and the movement of the ions (Dhillon et al. 2012). The synthesis of nanoparticles with a greener approach requires temperatures less than 100 °C or ambient temperature. The effect of temperature in the synthesis of fungal based nanoparticles was studied by Fayaz et al. (2009). They found that increase in temperature of the reaction results in decrease of the nanoparticle size but increase in monodispersity. Incubation time is also another important factor affecting the synthesis of fungal nanoparticles. The time period in which the reaction medium incubates greatly enhances the type of nanoparticle synthesized and the quality (Darroudi et al. 2011). The incubation time might occur in different ways such as the particles may aggregate because they are stored for a longer period of time; therefore, the potential is affected (Baer 2011).

4.4 Strategies for Mycosynthesis of Nanoparticles

There are two widely used approaches or strategies for the synthesis of nanoparticles. It can be synthesized using the top-down approach and bottom-up approach. In top-down method, physical methods, like diffusion, thermal decomposition, irradiation, etc., are employed. Nanoparticles are fabricated by using biological approach like biological entities and chemical approach like chemical reduction, seeded growth method, electrochemical synthesis and polyol synthesis method in bottom-up approach (Tikariha et al. 2012). In top-down approach, etching and machining techniques are employed in top-down approach where the large materials are ruptured down slowly to nanosized materials (Lengke et al. 2011). The synthesis of fungal nanoparticles is a bottom-up approach. The main reaction of mycosynthesis of nanoparticles resembles bottom-up approach because it includes substrate oxidation or reduction resulting to colloidal structures. The possibilities to a greater degree for acquiring nanostructures such as nanotubes, nanorods, nanowires, nanocubes, nanosheets, etc. with better uniformity in chemical composition in bottom-up approach make more advantageous than other approaches. This is due to this approach, which is mainly steered by reduction of Gibbs free energy that brings those synthesized nanomaterials or nanostructures to a state closer to thermodynamic equilibrium state (Kashyap et al. 2013).

4.5 Applications of Mycosynthesis of Nanoparticles in Agriculture

Nanotechnology is a fast-developing industry which has an impact in the field of agriculture. Fungus-based synthesis of nanoparticles has attracted the researchers due to their enormous applications in various fields. Some of the applications in various fields of agriculture are illustrated below.

4.5.1 Nanofungicides

The common plant pathogens are fungi compared to viruses and bacteria. These plant pathogens such as species of *Aspergillus*, *Fusarium* and *Phytophthora* can be used as a nanomaterial for the synthesis of nanoparticles (Yadav et al. 2015). Kim et al. (2012) reported a silver nanoparticle of nanosize which can be used as an effective antifungal agent in the treatment of different plant pathogens. In vitro assay was performed on petri dish. They used 18 plant pathogenic fungi for treating the silver nanoparticles on malt extract agar, potato dextrose agar and cornmeal agar plates. The fungal inhibition was calculated to evaluate the antifungal activity of silver nanoparticles against the pathogens. The results revealed that silver nanoparticles possess antifungal properties against these plant pathogens at different levels. In vitro as well as field conditions trial was conducted by Lamsal et al. (2011). They demonstrated the effects of silver nanoparticles using *Colletotrichum* species and pepper anthracnose disease. Silver nanoparticle solution at different concentrations, viz. 10, 30, 50 and 100 ppm, was used. In vitro assay was performed. The maximum inhibition rate was at 100 ppm silver nanoparticles solution with a percentage of 93.50%, while the lowest inhibition was found to be 11.33% at 10 ppm. In the case of field trial analysis, an experiment was conducted before and after the pepper was infected. Two positive controls, i.e. commercial fungicide NSS-F and chemical fungicide Feneri, were used. The leaves of the plants were treated with silver nanoparticles 3–4 weeks before and after the outbreak of the disease. After the disease outbreak treatment, results were analysed 1 week after the final treatment. Before the disease outbreak treatment, results were obtained 4 weeks after final treatment. Each experiment was performed in triplicates. Moreover, after the disease outbreak treatments, disease incidence was higher compared to before the disease outbreak treatments with NSS-F 72.1% and Fenari 63.4%. The lowest disease incidence was noticed on plants treated with 50 ppm silver nanoparticles before the disease outbreak with 9.7%, whereas the highest disease incidence was observed on plants treated with NSS-F after the disease outbreak with 72.1%. The results showed that before the outbreak of the disease, silver nanoparticle treatment was applied which suppressed the pathogen attack. Park et al. (2006) also studied the efficiency of nanosized silica-silver in controlling plant pathogenic microbes. Silica-silver nanoparticles were prepared, and antifungal activity was performed against *Rhizoctonia solani*, *Botrytis cinerea*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides* and *Pythium ultimum*. Antifungal effect was performed on powdery mildew in the field. The results suggest that since silver and silica are nontoxic and safe for human health, the cost is much lower than the commercial fungicide. This nanoformulation is highly useful for managing different fungal plant diseases (Bhattacharyya et al. 2016).

4.5.2 Nanopesticides

Plant diseases have reduced agriculture production. Various methods have been employed in combating the different diseases of plants such as natural or artificial methods. Excessive use of pesticides can cause environmental hazards. Therefore, scientists have been investigating for the replacement of chemical-based pesticides (Kim et al. 2012). Due to its durability, and high efficacy, nanopesticides represent the next-generation pesticides (Bhattacharyya et al. 2016). Nanopesticide can be prepared in two ways: organic ingredients which are polymers and inorganic, i.e. metal oxides (Yadav et al. 2015). Bramhanwade et al. (2016) studied a stable copper nanoparticle and their fungicidal activity against three crop pathogenic fungi. Cetyltrimethylammonium bromide and copper nitrate were used to synthesize stable copper nanoparticles at room temperature. The antifungal effect was determined against three crop pathogenic *Fusarium* spp., i.e. *F. oxysporum*, *F. culmorum* and *F. equiseti*, and found to exhibit significant antifungal activity. Therefore, copper nanoparticles can be used as antifungal agents.

4.5.3 Nanofertilizers

Nanofertilizers are those vital nutrients for the growth of the plants for improvement in their production and supporting their growth (Liu and Lal 2015; Prasad et al. 2014, 2017). Excessive consumption and continuous use of chemical fertilizers decrease the fertility of soil and in the crop production. Therefore, nanofertilizers can replace in regaining and protecting the fertility of soil. The use of nanofertilizers leads to an increase in nutrient efficiencies and reduces toxicity of the soil (Prasad et al. 2014, 2017). An eco-friendly and low-cost method was reported by Tarafdar et al. (2014). Zinc nanoparticle was employed as a nanofertilizer in pearl millet *Pennisetum americanum* L. for enhancing crop production. Zinc oxide solution reacted with the fungus *R. bataticola* for 62 h resulted in the extracellular synthesis of high monodispersed zinc nanoparticles with an average size of 18.5 nm as confirmed by transmission electron microscopy. In order to determine the effect of the synthesized zinc nanoparticle as a nanofertilizer, seeds of pearl millet were sown at 3 cm depth in the field. The field experiment was conducted with three treatments such as control, i.e. without any treatment, nanosize and normal size zinc oxide. After 2 weeks of germination, foliage was sprayed with the normal size zinc oxide and nanozinc. Results were observed after 4 weeks of spray in which significant improvement was observed in shoot length, root area and root length. In addition, the chlorophyll content, dry biomass plant, total soluble leaf protein, dehydrogenase, and enzyme activities of acid phosphatase were also estimated in 6-week-old plants. In another study conducted by Pandey et al. (2010), zinc oxide nanoparticle served as an efficient nanofertilizer for zinc, which is an essential nutrient for the growth of the plants. Singhal et al. (2017) 'reported that nano-embedded fungus' formed by impact of synergistic association of ZnO-nanorods and fungus *Piriformospora indica* DSM 11827, for growth of *Brassica oleracea* var. botrytis (Broccoli). Hydrothermal method was employed for the synthesis of zinc oxide nanoparticles and was characterized by powder X-ray diffraction, and field emission electron microscopy provided the size range with a diameter of 20–30 cm and is of spherical shape. Zinc oxide nanoparticle was used during the root growth and seed germination of *Cicer arietinum* which resulted in an increased level of Indole acetic acid in the roots, therefore bringing out an increase in the rate of plant's growth.

4.6 Conclusion and Future Perspectives

Mycosynthesis of nanoparticles has attracted a wide attention among researchers due to their desirable characteristics and nontoxicity. The chapter illustrates the various reports exhibited by the researchers on the extracellular and intracellular synthesis of fungal nanoparticles. The different parameters that affect nanoparticles mycosynthesis have been discussed. Moreover, the applications in the field of agriculture such as nanofungicide, nanopesticide and nanofertilizer are also exemplified. An extensive research of myconanotechnology in the field of agriculture will improve in the growth of the plants and crop protection. In-depth studies are essential on the mechanism of mycosynthesis of nanoparticles at the molecular and cellular level.

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