Myconanotechnology in agriculture: a perspective

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Received: 10 June 2012/Accepted: 13 September 2012/Published online: 22 September 2012 © Springer Science+Business Media Dordrecht 2012

Abstract Myconanotechnology is an emerging field, where fungi can be harnessed for the synthesis of nanomaterials or nanostructures with desirable shape and size. Though myconanotechnology is in its infancy, potential applications provide exciting waves of transformation in agriculture and fascinate microbiologists and other researchers to contribute in providing incremental solutions through green chemistry approaches for advancing food security. In this article, we provide a brief overview of the research efforts on the mycogenic synthesis of nanoparticles with particular emphasis on mechanisms and potential applications in agriculture and allied sectors.

Keywords Mycosynthesis · Nanoformulation · Nanowires · Nanofactories · Quantum dots

Introduction

Nanotechnology serves as a platform to harness the benefits of unique properties of atoms and molecules at the nano scale (Singh et al. 2011). The synthesis of nanomaterials with specific composition, size and of distinct properties has expanded the scope of their applications in myriad of industries including agriculture, textile and food (Sharon et al. 2010; Sastry et al. 2011). Conventional techniques of nanoparticles (NPs) synthesis that usually employ atomic, molecular and particulate processing in vacuum or in a liquid medium are capital intensive (Mandal et al. 2006; Anandan use (Pugazhenthiran et al. 2009). The non-toxic and environmentally benign procedures for the synthesis of such materials based on green chemistry and biological processes (Bhattacharya and Gupta 2005) needs to be developed. Consequently, researchers have used biological processes that provide excellent control over particle size (Korbekandi et al. 2009) via quantum confinement (Sastry et al. 2003). This resulted in relatively new, largely unexplored and rapidly growing area of myconanotechnology (Youtie et al. 2008; Gade et al. 2010; Kannan and Subbalaxmi 2011) which is evident from the large number of patents related to myconanotechnology in United States Patent and Trademark Office (USPTO) database in the last few years (Fig. 1). In general, myconanotechnology research, to date, focused only on developing manufacturing protocols of nanoparticles using fungi. However, recent technological advances that make better understanding of physicochemical and optoelectronic properties, and organization of nanoscale structures into predefined 1-D (nanorods), 2-D or 3-D (nanowires) superstructures (Bigall and Eychmüller 2010) have opened the door to new functionalities and applications of nanoparticles in agriculture, textile and food industry.

et al. 2008) and inefficient in terms of materials and energy

To synthesize the mycogenic nanomaterial such as nanowires, nanofilters, nanosensors, nanofibrous mats and quantum dots (QDs) as nanofactories, to suppress plant pathogen, targeted delivery, interactive agrochemicals as pesticides, nanocomposites material for food packaging and extensive nano-surveillance using crop sensing and nano-enabled diagnostics, it is necessary to accentuate on the mechanisms and approaches for fabrication of myconanoparticles. This review summarizes current research efforts, new opportunities and challenges for mycogenic nanoparticles synthesis and their potential applications in agriculture and related sectors.

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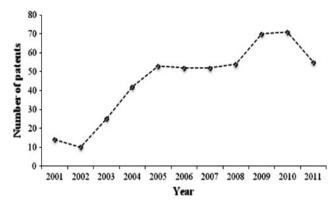
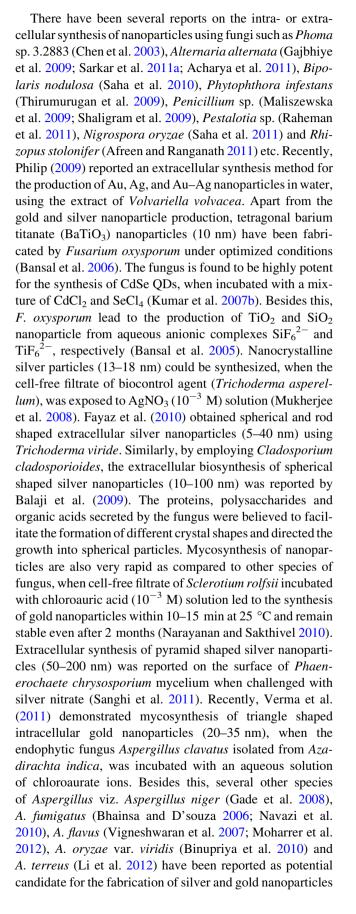


Fig. 1 Growth of myconanotechnology evident from number of patents in USPTO database up to August, 2011

Fungi as natural factories for biosynthesis of nanoparticles

The mycogenic synthesis of nanoparticles, an important aspect of myconanotechnology leads to an exciting new and pragmatic interdisciplinary science with considerable potential due to wide spectrum and diversity in fungi (Rai et al. 2011). Recently, fungi have gained much importance, as the extracellular secretion of enzymes has an added advantage in the downstream processing and handling of biomass (Gade et al. 2008), when compared to the bacterial fermentation process which involves use of sophisticated instruments to obtain clear filtrate from the colloidal broth (Sastry et al. 2003). Moreover, fungi are excellent secretors of protein compared to bacteria and actinomycetes, results into higher yield of nanoparticles (Rai et al. 2009). Several fungi have already been exploited for the synthesis of silver, gold, zirconium, silica, titanium, iron and platinum nanoparticles (Krumov et al. 2009; Gade et al. 2010). Fungi have a number of advantages for both intra- or extracellular synthesis of nanoparticle over other organisms including plants (Narayanan and Sakthivel 2010). Due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization (Vaidyanathan et al. 2009). Fungal mycelia can withstand flow pressure, agitation and other conditions in bioreactors or other chambers compared to plants and bacteria (Saha et al. 2010). Many of the enzymes secreted by fungi are capable of reducing metal ions quickly through non-hazardous processes and allow a controlled synthesis of nanoparticles with well defined size and shape (Moaveni et al. 2011). Moreover, large scale extracellular secretions of their reductive proteins helps in outside precipitation of nanoparticles, minimizes the unnecessary cellular components and hence, aiding direct use in various applications. Therefore, fungi could be regarded as natural 'biofactories' for biosynthesis of nanoparticles.





with desired shape and size. The recent research on synthesizing nanoparticles using fungal biosystem is summarized in Table 1.

Strategies for mycosynthesis of nanoparticles

There are two strategies for the synthesis of metal nanoparticles: a bottom-up (self-assembly) and a top-down (Marchiol 2012). Bottom-up strategy refers to the construction of a structure atom-by-atom, molecule-by-molecule, or by self organization. In the top-down approach, a suitable starting material is reduced in size using physical or chemical means. Cutting, grinding and etching are typical fabrication techniques, which have been developed to work on the nano scale (Singh et al. 2011). Mycosynthesis of nanoparticles is a kind of bottom-up approach (Fig. 2), whereby the main reaction occurring involves reduction/ oxidation of substrates, giving rise to colloidal structures (Moghaddam 2010). Fungal enzymes or metabolites with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles (Zhang et al. 2011). Mycoreduced metal atoms undergo nucleation with subsequent growth, leading to the generation of nanostructures. The production of functional nanometer sized objects and semiconductor QDs are good example of bottom-up approach (Saravanan et al. 2008). An advantage of the bottom-up approach is the better possibilities to obtain nanostructures (nanorods, nanocubes, nanotubes, nanowires and nanosheets etc.) with minor defects and more homogeneous chemical compositions (Thakkar et al. 2010). This is because the bottom-up approach is driven mainly by the reduction of Gibbs free energy, so that such synthesized nanostructures/nanomaterials are in a state closer to a thermodynamic equilibrium state (Behari 2010).

Mechanism of mycosynthesis of nanoparticles

At present the mechanism for nanoparticles(s) fabrication by fungi is not clear, according to speculation by various researchers, the hypothetical representation for the mycosynthesis of nanoparticles is depicted in Fig. 3. The ability of microorganisms to grow in the presence of high metal concentrations might result from specific mechanisms of resistance and their adaptability in extreme conditions. Efflux systems, alteration of solubility, and toxicity by changes in the redox state of the metal ions, extracellular complexation or precipitation of metals, and the lack of specific metal transport systems are some of the mechanisms possessed by microorganisms (Dhillon et al. 2012). Thus, it might be speculated that in the mycogenic synthesis of metal nanoparticles, the fungus mycelium is

exposed to the metal salt solution that prompts the fungus to produce enzymes and metabolites for its own survival. In this process the toxic metal ions are reduced to the nontoxic metallic nanoparticles through the catalytic effect of the extracellular enzymes and metabolites of the fungus.

The intracellular synthesis of nanoparticles by bioreduction, initiated with trapping of metal ions over fungal cell surface, probably due to the electrostatic interaction with the enzymes/proteins present on the fungal cell wall. In the next step, the metal ions are reduced by the enzymes within the cell wall, which leads to the aggregation of metal ions and formation of nanoparticles (Mukherjee et al. 2001b). There are the possibility that some metal ions may diffuse through cell wall and are reduced by the enzymes present on the cytoplasmic membrane and cytoplasm (Sastry et al. 2003). Further, Durán et al. (2005) conducted nitrate reductase assay to explore the possible role of reductase activity or electron shuttle quinones or both in the extracellular mycosynthesis of nanoparticles. They concluded that the enzyme reductase is responsible for the reduction of Ag⁺ ions and the subsequent extracellular formation of silver nanoparticles. The role of nitrate reductase in the synthesis of nanoparticles was also studied by Kumar et al. (2007b). The enzyme α -NADPH-dependent nitrate reductase was isolated from F. oxysporum and used for in vitro synthesis of silver nanoparticles. The spectra of reaction mixture showed strong surface plasmon resonance at 413 nm which intensified with time, while the absence of absorption band at 413 nm in the absence of enzyme clearly depicted that the reduction of silver involves enzymatic reduction of nitrate to nitrite. Thus, indicating that the synthesis of silver requires the reduction of NADPH to NADP⁺ and the hydroxyquinoline probably acts as an electron shuttle transferring the electron generated during the reduction of nitrate to Ag⁺ ions converting them to Ag° (Fig. 3). In accordance with the above studies, Ingle et al. (2008) conducted the nitrate reductase test using commercially available nitrate reductase discs to clarify the above assumptions. The colour of the disc turned reddish from white when challenged with fungal filtrate signifying the presence of nitrate reductase. Briefly, it can be concluded that the electrostatic interaction and specific enzyme(s) of fungi (e.g. NADPH dependent reductase enzyme and hydroxyquinoline, phytochelatin etc.) are major factors in the mycogenic synthesis of nanoparticles, even though the real mechanism of biosynthesis of nanoparticles is still unclear.

Factors affecting mycosynthesis of nanoparticles

Based on the studies of the nanoparticle mycosynthesis, optimum temperature, pH, exposure time to substrate,



Table 1 Use of fungi in the synthesis of nanoparticles and their mode of synthesis

Fungi	Nanoparticle(s)	Size (nm)	Morphology	Mode of synthesis	References
A. alternata	Ag	20-60	Spherical	Extracellular	Gajbhiye et al. (2009)
A. alternata	Se	30 ± 5	Spherical	Extracellular	Sarkar et al. (2011b)
A. alternata	Au	12 ± 5	Spherical	Extracellular	Sarkar et al. (2011a)
A. alternata	Ag	35-90	ND	Extracellular	Acharya et al. (2011)
A. clavatus	Ag	10-25	Spherical, hexagonal	Extracellular	Verma et al. (2010)
A. clavatus	Au	20-35	Triangular	Intracellular	Verma et al. (2011)
A. flavus	Ag	8.92 ± 1.62	ND	Intracellular	Vigeshwaran et al. (2007)
A. flavus NJP08	Ag	17 ± 5.9	Spherical	Extracellular	Jain et al. (2010)
A. flavus	Ag	7	Spherical	Extracellular	Moharrer et al. (2012)
A. fumigatus	Ag	5–25	Spherical, triangular	Extracellular	Bhainsa and D' Souza (2006)
A. fumigatus	Ag	7–19	Variable shapes	Extracellular	Navazi et al. (2010)
A. niger	Ag	15-20	ND	Extracellular	Gade et al. (2008)
A. oryzae var. viridis	Au	10-60	Triangle, pentagon, hexagon	Extracellular	Binupriyaa et al. (2010)
A. terreus	Ag	1–20	Spherical	Extracellular	Li et al. (2012)
Bipolaris nodulosa	Ag	10–60	Spherical, semi-pentagonal, hexahedral	Extracellular	Saha et al. (2010)
C. cladosporioides	Ag	10-100	Spherical rods and triangular	Extracellular	Balaji et al. (2009)
Cochliobolus lunatus	Ag	3–21	Spherical	Extracellular	Salunkhe et al. (2011)
Colletotrichum sp.	Au	20-40	Spherical	Extracellular	Shankar et al. (2003)
C. versicolor	Ag	25-75	Spherical	Extracellular	Sanghi and Verma (2009)
C. versicolor	CdS	100	Spherical	Extracellular	Chen et al. (2011)
F. acuminatum EII. & Ev.	Ag	4–50	Spherical	Extracellular	Ingle et al. (2008)
F. oxysporum 284	Au	20-40	Multishaped	Extracellular	Anitha and Palanivelu (2011)
F. oxysporum	Au	20–40	Spherical, triangular	Extracellular	Mukherjee et al. (2002)
F. oxysporum	Ag	5–50	ND	Extracellular	Senapati et al. (2004)
F. oxysporum	CdS	5–20	Spherical	Extracellular	Ahmad et al. (2002)
F. oxysporum	Ag	5–15	Quasi-spherical	Extracellular	Ahmad et al. (2003)
F. oxysporum	SrCO ₃	ND	Needle shaped	Extracellular	Rautaray et al. (2004)
F. oxysporum	Zirconia	3–11	Quasi-spherical	Extracellular	Bansal et al. (2005)
F. oxysporum	Si	5–15	Quasi-Spherical	Extracellular	Bansal et al. (2005)
F. oxysporum	Ti	6–13	Spherical	Extracellular	Bansal et al. (2005)
F. oxysporum	Ag	20–50	Spherical	Extracellular	Durán et al. (2005)
F. oxysporum	Magnetite	20–50	Quasi-spherical	Extracellular	Bharde et al. (2006)
F. oxysporum F. oxysporum	BaTiO ₃	4–5	Quasi-Spherical	Extracellular	Bansal et al. (2006)
F. oxysporum F. oxysporum		10–25	Multishaped	Extracellular	Kumar et al. (2007a)
	Ag		î .		
F. oxysporum	CdSe	9–15	Spherical Spherical	Extracellular	Kumar et al. (2007b)
F. oxysporum	Ag	5–60	Spherical	Extracellular	Mohammadian et al. (2007)
F. oxysporum	Bi ₂ O ₃	5–8	Quasi-Spherical	Extracellular	Uddin et al. (2008)
F. oxysporum	Ag	50	Spherical	Extracellular	Karbasian et al. (2008)
F. oxysporum	Pt	20–60	Triangle	Extracellular	Govender et al. (2009)
F. oxysporum F. oxysporum f. sp. lycopersici	Ag Pt	20–70 10–50	Multishaped Triangle, hexagons, square,	Extracellular Intra-/extra-	Pandiarajan et al. (2010) Riddin et al. (2006)
			rectangles	cellular	
Fusarium semitectum	Au, Au–Ag	18–80	Multishaped	Extracellular	Sawle et al. (2008)
F. semitectum	Ag	10–60	Spherical	Extracellular	Basavaraja et al. (2008)
Fusarium solani	Ag	3–8	Spherical	Extracellular	El-Rafie et al. (2010)
F. solani USM 3799	Ag	16.23	Spherical	Extracellular	Ingle et al. (2009)
Helminthosporum solani	Au	2–70	Rods, triangles, pentagons, stars, and pyramids	Extracellular	Kumar et al. (2008)
L. lecanii	Ag	45–100	Spherical	Extracellular	Namasivayam and Avimanyu (2011)
Neurospora crassa	Ag	11	Spherical	Intra-/extra- cellular	Castro-Longoria et al. (2011)



Table 1 continued

Fungi	Nanoparticle(s)	Size (nm)	Morphology	Mode of synthesis	References
N. crassa	Au	32	Spherical	Intra-/extra- cellular	Castro-Longoria et al. (2011)
N. oryzae	Ag	30–90	Spherical	Intra-/extra- cellular	Saha et al. (2011)
Penicillium sp.	Ag	16-40	Multishaped	Extracellular	Sadowski et al. (2008)
Penicillium brevicompactum WA2315	Ag	58.35 ± 17.88	Spherical	Extracellular	Shaligram et al. (2009)
P. fellutanum	Ag	5–25	Spherical	Extracellular	Kathiresan et al. (2009)
Penicillium sp.	Au		Spherical	Intracellular	Zhang et al. (2009)
Penicillium sp.	Ag	10-100	Spherical	Extracellular	Maliszewska et al. (2009)
Penicillium sp.	Ag	52-104	Multishaped	Extracellular	Hemath et al. (2010)
Penicillium sp.	Au	30–50	Spherical	Intra-/extra- cellular	Du et al. (2011)
Pestalotia sp.	Ag	10-40	Spherical	Extracellular	Raheman et al. (2011)
P. chrysosporium	Ag	5-200	Pyramidal	Extracellular	Vigneshwaran et al. (2006)
Phanerochaete chrysosporium	Au	10-100	Spherical	Extracellular	Sanghi et al. (2011)
Phoma glomerata	Ag	60-80	Spherical	Extracellular	Birla et al. (2009)
Phoma sorghina	Ag	120-160 × 30-40	Rods	Extracellular	Gade et al. (2011)
Phoma sp. 3.2883	Ag	71.06 ± 3.46	Spherical	Extracellular	Chen et al. (2003)
P. infestans	Ag	ND	ND	Extracellular	Thirumurugan et al. (2009)
Pleurotus sajor caju	Ag	5-50	Spherical	Extracellular	Nithya and Ragunathan (2009)
R. oryzae	Au	10	Multishaped	Extracellular	Das et al. (2009)
R. stolonifer	Ag	5-50	Spherical	Extracellular	Afreen and Ranganath (2011)
S. rolfsii	Au	25	Triangle, decahedral, and spherical	Extracellular	Narayanan and Sakthivel (2010)
T. asperellum	Ag	13-18	ND	Extracellular	Mukherjee et al. (2008)
Trichoderma harzianum	Ag	30-50	Spherical	Extracellular	Singh and Balaji (2011)
Trichoderma reesei	Ag	5-50	Multishaped	Extracellular	Vahabi et al. (2011)
T. viride	Ag	5-40	Spherical and rod-like	Extracellular	Fayaz et al. (2010)
Tricholoma crassum	Ag	5-50	Spherical, hexagonal	Extracellular	Ray et al. (2011)
Trichothecium sp.	Au	5-200	Triangle, hexagonal	Extracellular	Ahmad et al. (2005)
Trichothecium sp.	Au	ND	Spherical, rods and triangular	Intra-/extra- cellular	Ahmad et al. (2005)
V. luteoalbum	Au	≥10	Spherical, spheres and rods	Intracellular	Gericke and Pinches (2006)
Verticillium sp.	Magnetite	100-400	Cubo-octohedral	Extracellular	Bharde et al. (2006)
Verticillium sp. AAT-TS-4	Ag	25 ± 12	Spherical	Intracellular	Mukherjee et al. (2001a), Senapati et al. (2004)
Verticillium sp. AAT-TS-4	Au	20 ± 8	Spherical, quasi-hexagonal	Intracellular	Mukherjee et al. (2001a)
V. volvacea	Au, Ag, Au–Ag	20–150	Spherical, hexagonal	Extracellular	Philip (2009)

ND not determined

biomass, presence of specific enzyme and substrate concentration were suggested as major parameters to affect particle size, shape and monodispersity of nanoparticle(s). Karbasian et al. (2008) employed response surface methodology to investigate the effect of pH, temperature, agitation rate, incubation time, silver salt concentration and weight of fungal biomass on the formation of silver nanoparticles. They obtained spherical shaped silver nanoparticles (50 nm) by dipping *F. oxysporum* in silver nitrate (3 mM; pH 6.0) solution and incubated at 25 °C with 180 rpm agitation for 96 h.

The influence of metal ion concentration on the synthesis of nanoparticles employing *Penicillium fellutanum* suggested that high concentration would hamper the formation of nanoparticles. The particle size and monodispersity of the particles diverge from the desire nanosize range at high silver ion concentration (Kathiresan et al. 2009). Similar to chemical reaction, the concentrations of reactants decide the reaction extent and affects the particle size and monodispersity. One example is gold nanoparticle synthesis using *Verticillium luteoalbum*. The results revealed that when AuCl₄ concentration was below



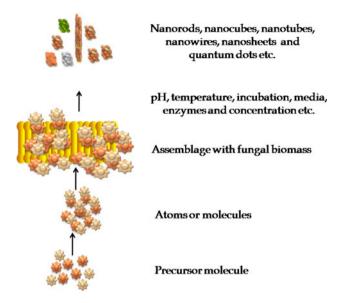


Fig. 2 Bottom-up strategy for the mycogenic synthesis of nanoparticles start with one or more precursor molecules, which undergo certain processes that result in well-organized assemblage of atoms and molecules using fungus biomass. Examples of bottom-up approaches include systems that self-assemble and triggered by minor alterations in a chemical or physical condition such as change in pH, temperature, incubation, presence of specific enzyme, media, concentration and stabilising agent etc

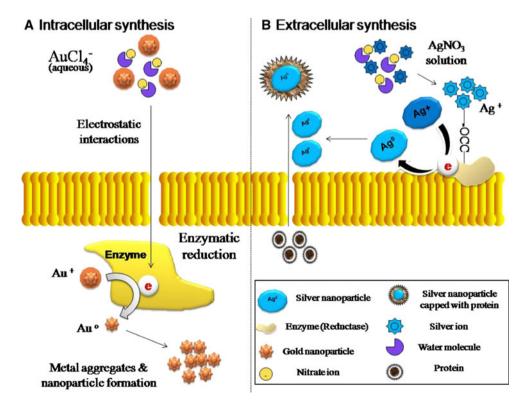
 $500 \text{ mg } 1^{-1}$, the particle size was narrow ($\sim 20 \text{ nm}$) and uniform, however, when the concentration was above this, the particle size increased with concentration of AuCl^{-4} , but varied from 50 nm to several hundred nanometers. In

Fig. 3 Putative mechanisms of nanoparticle synthesis using fungal biosystems. During intracellular synthesis (a), metal ions (e.g. gold) initially bind on the fungal cell surface through electroststaic interation. The adsorbed metal ions are then reduced by positively charged groups in enzymes present in the cell wall, leading to their aggregations and the formation of nanoparticles. In case of extracellular synthesis (b), the exposure of metal ions to fungus resulted in the releaseof reductase enzyme (such as nitrate reductase) and subsequent formation of highly stable nanoparticles (e.g. silver) in solution

addition, massive particle aggregate was found on the cells (Gericke and Pinches 2006).

pH is an important parameter having pronounced effects on the synthesis of nanoparticles. In an attempt to reveal the role of pH in fabrication of nanoparticles, Gericke and Pinches (2006) demonstrated the changes in the shapes of the crystals with varying pH. They found that the nanoparticles produced by V. luteoalbum at pH 3 were predominantly spherical in shape (<10.0 nm), and obtained bigger nanoparticles with well-defined shapes such as hexagons, triangles, rods, and spheres at pH 5.0. However, at pH 7 and 9, the particles formed included small spherical particles as well as bigger particles with irregular and undefined shapes. Moreover, the number of particles produced per cell was lower at pH 9 than at pH 7. In another study conducted by Sanghi and Verma (2009), Coriolus versicolor was used as fungal biosystem to study the pronounced effects of pH on nanoparticles fabrications. They suggested that reduction reaction of metallic ions was sensitive to pH. Under the alkaline conditions (pH 10.0) the time taken for the production of silver nanoparticles was reduced from 72 to 1 h.

Temperature plays a promising role in controlling fungus activities and ions movement (Dhillon et al. 2012). Therefore, it can be supposed that temperature exerts marked effects on the growth of fungus as well as on metal uptake from the surrounding environment. As a result, the rate of formation of the nanoparticle(s) was directly influenced by the incubation temperature (Gericke and Pinches





2006). They showed that at the lower temperatures, the majority of NPs formed after 1 h exposure to gold solution were spherical (<10 nm) in shape. Further increasing the incubation period up to 24 h led to a decrease in the number of smaller particles whereas, the number of larger particles displaying well-defined shapes increased. At 50 °C, no difference was detected in the size and morphology of particles produced after 1 and 24 h exposure to gold and very few small spherical particles were present. It is apparent from this study that the size of the nanoparticles to a large extent can be controlled by operating at low temperatures, although it would allow particle formation at a slower rate.

The use of specific enzymes secreted by fungi is extremely promising for the synthesis of predefined nanoparticles of different chemical compositions, shapes, and sizes. It is believed that these enzymes offer advantages for in vitro synthesis of nanoparticles using the fungal cell, since it would eliminate the need to harvest the intracellular nanoparticles and further optimization of the synthetic process. In one of the study, Ahmad et al. (2003) noticed that the silver nanoparticle was not synthesized in the presence of F. moniliforme but it was formed when F. oxysporum was present in the silver ion solution. Protein assay of the two fungi exhibited that a specific reductase enzyme (NADH-dependent reductase), was produced only by F. oxysporum, whereas the other reductases produced by the two fungi were the same (Ahmad et al. 2003). It clearly indicates that NADH-dependent reductase is probably resulting in the production of nanoparticles. Similarly, Bansal et al. (2006) reported that fungal enzymes secreted from F. oxysporum selectively bioleached silicates, present in zircon sand, into silicic acid and, subsequently into silica nanoparticles. Exposure of F. oxysporum to an aqueous solution of K₂ZrF₆, resulted in its protein-mediated extracellular hydrolysis, to form crystalline zirconia nanoparticles. However, with few exceptions it is not fully established that what type of different enzymes are involved in mycogenic synthesis of nanoparticles. Besides enzymes, identification of the nature of capping surfactants/peptides/proteins and other stabilizing agents is also equally important. The known surface chemistry of the mycogenic nanoparticles would then lead to the possibility of genetically engineering fungi to over-express specific reducing molecules and capping agents, and thereby, control the size and shape of the nanoparticles.

Briefly, synthesis parameters have expressed their significant contribution on the nanoparticles production. So far, the better control on the particle size, shape and monodispersity is still unknown. There is a big challenge on the mycosynthesis of nanoparticles and different parameters discussed here need to be optimized. As, minimum time, miniaturization and non-hazardous processes

are key factors for the acceptance and large scale in vitro synthesis of nanoparticle using fungal systems (Kumar et al. 2007a), therefore, further study on type of fungus, culture growth stage, growth medium, synthesis conditions, pH, substrate concentrations, source compound of target nanoparticle, temperature, reaction time, agitation, agent materials participation and the presence of non function ions etc. should be done to explore the control of particle size, shape and monodispersity.

Myconanotechnology in agriculture

Nanotechnology is a new, fast-developing industry, posing substantial impacts on agriculture and allied sectors that likely will produce myriads of nanostructured materials (Mandal et al. 2006; García et al. 2010; Gade et al. 2010). Although, fungus mediated synthesis of nanoparticles and their wide array of applications (Table 2) have recently attracted the attention of researchers towards myconanotechnology. Some of the prominent applications in various sectors of agriculture have been described in following sections.

Antimicrobial nanomolecules

Myconanotechnology represents a new paradigm in the development of antimicrobial nanomaterials. It has been shown that extracellularly produced silver nanoparticles using F. oxysporum, can be incorporated in several kinds of materials such as cloths. These cloths with silver nanoparticles (1.6 nm) are sterile and can be useful to prevent infection with pathogenic bacteria such as Staphylococcus aureus (Durán et al. 2007). Silver nanoparticles (1–10 nm) attach to the surface of cell membrane of these pathogenic bacteria and drastically disturb its proper function like respiration and permeability (Morones et al. 2005). Silver nanoparticles (45-100 nm) synthesized by Lecanicillium lecanii, were coated on the bleached cotton fabrics using acrylic binder and anti bacterial properties of coated fabrics were noticed against S. aureus and Escherichia coli (Namasivayam and Avimanyu 2011).

The use of silver nanoparticles (4–8 nm) as an alternative to pesticides for the control of sclerotia forming phytopathogenic fungi was also investigated (Min et al. 2009) and found that the silver nanoparticles, which have high surface area and high fraction of surface atoms, have more antimicrobial effect compared to the bulk silver. A microscopic observation revealed that hyphae exposed to silver nanoparticles (4–8 nm) were severely damaged, resulting in the separation of layers of hyphal wall and collapse of hyphae. Extracellular synthesis of gold



Table 2 Potent applications of myconanoparticles in agriculture and allied sectors

Fungi	Nanoparticle	Applications	References
A. alternata	Ag	Enhancement in antifungal activity of fluconazole against <i>P. glomerata</i>	Gajbhiye et al. (2009)
A. niger	Ag	Antibacterial activity	Gade et al. (2008)
A. niger	Ag	Wound healing activity	Sundaramoorthi et al. (2009)
Aspergillus species	Ag	Antimicrobial activity	Saravanan (2010), Saravanan and Nanda (2010)
F. acuminatum	Ag	Antibacterial activity	Ingle et al. (2008)
F. oxysporum	Ag	Textile fabrics	Durán et al. (2007)
F. oxysporum	CdS	Live cell imaging and diagnostics	Kumar et al. (2007b)
F. solani	Ag	Textile fabrics	El-Rafie et al. (2010)
L. lecanii	Ag	Textile fabrics	Namasivayam and Avimanyu (2011)
P. infestans	Ag	Antibacterial activity	Thirumurugan et al. (2009)
R. oryzae	Au	Water hygiene management	Das et al. (2009)
T. crassum	Ag	Antimicrobial activity	Ray et al. (2011)
T. viride	Ag	Vegetable and fruit preservation	Fayaz et al. (2009)

Source: Modified after Gade et al. (2010)

nanoparticles (10 nm) using Rhizopus oryzae employed for the generation of nanogold-bioconjugate (NGBC) structure (Das et al. 2009). The designed nanostructures showed strong adsorption capacity and have been successfully utilized to obtain water, free from pathogens and pesticides. Kim et al. (2008) studied the antifungal effect of double capsulized nano silver (1.5 nm) solution against rose powdery mildew caused by Sphaerotheca pannosa var rosae. The nano silver (1.5 nm) solution was diluted up to 10 ppm and sprayed at large area infected 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. Recently, Aguilar-Méndez et al. (2010) evaluated the antifungal activity of the silver nanoparticles (5-24 nm) on Colletotrichum gloesporioides, which causes anthracnose in a wide range of fruits, such as apple, avocado, mango, papaya, etc. They reported significantly delayed growth of C. gloesporioides in the presence of silver nanoparticles. Therefore, nano silver could be an alternate of fungicide to manage plant diseases.

Nanowires

Biological synthesis of nanowires using fungi is very useful for miniaturization of electronic devices and their manufacturing in large quantity would be of great importance in the field of microelectronics, optoelectronics and nanoscale electronic devices that can be useful in precision and protected agriculture. Sugunan et al. (2007) reported a simple and efficient biological method for synthesis of gold microwires using *Aspergillus* and *Neurospora*. These fungi

were exposed for a week to gold nanoparticles (15 nm) that were surface functionalized with glutamate, aspartate and polyethylene glycol. This self organization is driven by fungal physiology of absorption of un-reacted precursors from the reduction reaction for synthesis of gold nanoparticles, as nutrients. The fungi get coated by gold particles (15 nm) while consuming the food in the solution and resulted in the assembly of the gold microwires (Sugunan et al. 2007). The method is expected to be applicable to the synthesis of silver and platinum nanowires as He et al. (2008) showed the synthesis of networked nanowires with an extract of Rhodopseudomonas capsulata. Rehman et al. (2011) devised a simple method to decorate growing fungal hyphae (A. niger) with high load of gold nanoparticles, which were initially produced using aqueous tea extract as a sole reducing/stabilizing agent. Heat treatment of these hybrid materials led to the formation of porous gold microwires. Furthermore, it is postulated that the nanowires based paper can be used to cleanup oil, organic pollutants in water and soil sediments and might be helpful for improving irrigation water quality in agriculture. Moreover, by altering the DNA sequence of the genes that encode for microbial nanowires, it may be possible to produce nanowires with different properties and functions (Reguera et al. 2005). Recently, Yoo et al. (2011) combined a patterned Au nanowire (NW)-on-film surfaceenhanced resonance raman scattering (SERRS) sensor with an exonuclease III-assisted target DNA recycling reaction for ultrasensitive and multiplex identification of pathogenic fungi. Using similar strategy, Kang et al. (2012) devised single-step multiplex detection system for detection of toxic metal ions by SERRS sensor and explained its utility



for rapid and quantitative detection of mercury (Hg²⁺), silver (Ag⁺), and lead (Pb²⁺) ions in same solution. Thus, nanowires might be tuned for devising effective monitoring and diagnostic tools for the detection of toxic metals, pathogens, and chemicals in agriculture and agro-products (Roach 2006; Chau et al. 2007; García et al. 2010).

Nanofibrous mats

Nanofibrous material offers unique features and has generated many interesting and useful applications. Due to high surface area and porous structure of the electrospun fibers, they serve as an ideal material for wide applications in numerous fields such as biosensors, tissue engineering, photonics, nanocomposites, catalysts and antimicrobial materials, protective clothes, and drug delivery (Bhardwaj and Kundu 2011). Spasova et al. (2011) prepared nanofibrous mats containing chitosan and T. viride spores by electrospinning. It was observed that T. viride placed at 28 °C grows much faster and fights for space and nutrients against Fusarium sp. and Alternaria sp. In addition, T. viride produces extracellular hydrolytic enzymes which directly attack the pathogen and destroying their cell walls. The spores incorporated into the fibrous mats are viable and the grown T. viride preserves its ability to inhibit the growth of test phytopathogens.

Quantum dots (QDs)

Recent advances in the field of luminescent nanocrystals have led to a new frontier of research in fluorescent labelling by QDs with bio-recognition molecules (Shao et al. 2011). ODs are nearly spherical, fluorescent nanocrystals composed of semiconductor material that bridge the gap between individual atoms and bulk semiconductors solids (Drummen 2010). Owing to their intermediate size, which is typically between 2 and 8 nm in diameter, they posses several advantages over conventional organic flurophores. They are most efficient in luminescence compared to the organics dyes and their emission spectra are narrow, symmetric and tuneable. They show excellent photostability (Müller et al. 2006), depending on quantum dot particle size and monolayer structure, with appropriate choice of both particle size and ligand structure required for intracellular stability (Zhu et al. 2011). Contrary to a number of chemical methods used in the synthesis of QDs that are often energy intensive, employ toxic chemicals, and require higher temperatures, the synthesis by mycological systems has been characterized by processes that occur at ambient temperature (25 \pm 1 °C) and pressure (1 millibar). The synthesis of highly luminescent CdSe QDs at room temperature (26 \pm 1 °C) has been well documented (Kumar et al. 2007a), when F. oxysporum was incubated with a mixture of CdCl2 and SeCl4. More recently, Bao et al. (2010) also demonstrated a simple and efficient biosynthesis method to easily harvest cadmium telluride (CdTe) QDs (2-3.6 nm) with tunable fluorescence emission using yeast cells. Furthermore CdTe QDs are naturally capped with proteins in the biosystem, and show excellent water solubility, great stability and highly fluorescent biocompatibility. However, till now there are no report of application of fungus derived QDs in agriculture, food and textile industry. Recently, Bakar et al. (2011) developed an optical sensor for the detection of pesticides (Siven 85 % wettable powder) in water using ZnCdSe QD films. Hence, we expect that in coming years, fungus derived QDs, which offer advantages in terms of cost effectiveness, non-toxicity and reproducibity at room temperature and pressure over conventional chemical and physical approaches will emerge as magic bullets in the field of live cell imaging, plant disease diagnostic, immune-histochemistry and in fluorescence in situ hybridisation (FISH) experiments.

Nanoformulation(s)

Nanoparticle formulation(s) of biopesticides have been proposed to produce a better spatial distribution of the pesticides on leaf surfaces, which provides better efficiency (Liu et al. 2008). Fungus derived nanoparticles are well studied for drug delivery and sustained release in medical science (Narayanan and Sakthivel 2010) but not extensively in the agricultural sciences. Boehm et al. (2003) obtained stable polymeric nanospheres with 3.5 % encapsulation rate and hence showed significant improvement in the bioavailability of insecticide (RPA 107382) to plants. They also performed biological studies on cotton plants infested with aphids to estimate the direct contact efficacy of nanosphere formulations on insects. The nanosphere formulations performed better than the reference to manage the infestation. It has been reported that nanoparticles loaded with garlic essential oil is efficacious against Tribolium castaneum Herbst (Yang et al. 2009). Also, it is known that aluminosilicate filled nanotube can stick to plant surfaces while nano ingredients of nanotube have the ability to stick to the surface hair of insect pests and ultimately enters the body and influences certain physiological functions (Patil 2009). Goswami et al. (2010) also studied the application of solid and liquid formulations of silver, aluminium oxide, zinc oxide and titanium dioxide nanoparticles in the control of rice weevil and grasserie disease in silkworm (Bombyx mori) caused by Sitophilus oryzae and baculovirus BmNPV (B. mori nuclear polyhedrosis



virus), respectively. After 7 days of exposure, 95 and 86 % mortality were observed with hydrophilic and hydrophobic formulations of silver based nanoformulations and nearly 70 % of the insects were killed when the rice was treated with lipophilic formulations of SNP. Recently, initiative for the preparation of bio-nanoformulations was also undertaken by Mukherjee et al. (2008). They synthesized silver nanoparticles (13–18 nm) with well-defined morphology and stability over several months by using *T. asperellum*. Similar attempts were made by several other workers by using *Trichoderma* strains (Vahabi et al. 2011), but their potential as biopesticide is still to be realised.

Smart delivery systems

A very interesting application of nanoparticles in agriculture is their use as smart delivery systems (González-Melendi et al. 2008). The great potential of using nanomaterial as delivery systems to specific targets in living organisms was first explored for medical uses (Kukowska-Latallo et al. 2005). In plants, the same principles can be applied for numerous applications, in particular to manage insect pest and diseases. Nanoparticles tagged to agrochemicals or other substances could reduce the damage to non-target plant tissues and the amount of chemicals released into the environment (Nair et al. 2010). To explore the benefits of nanotechnology to agriculture, the first stage is to work out the correct penetration and transport of the nanoparticles into plants. Nanofiber arrays which can deliver genetic material to cells quickly and efficiently have potential applications in slow delivery of agrochemicals and site targeted delivery of various macromolecules needed for improved plant disease resistance, crop engineering and environmental monitoring (Moaveni et al. 2011). Gene transfer by bombardment of DNA-absorbed gold particles has been successfully used to generate transgenic plants in a species-independent manner (Christou et al. 1988). Recently, Torney et al. (2007) reported the efficient delivery of DNA and chemicals through silica nanoparticles internalized in plant cells, without the requirement of specialized equipment. Small interfering RNA (siRNA) delivery can be monitored by a novel method based on nano-device that combines unmodified siRNA with semiconductor QDs as multicolor biological probes. Cotransfection of siRNA with QDs using standard transfection techniques has led to the formation of photo stable fluorescent nanoparticles that helps in tracking the delivery of nucleic acid, the degree of transfection in cells and also in purifying homogeneously silenced subpopulations (Elumalai et al. 2011). Thus, smart delivery systems at a nanoscale serve as carriers and provide real time chemical detection and decision taking ability for selfregulation. Although, attempts are ongoing in this track using mycomimetic system but still we have wait to visualise their operation in farming.

Nano-enabled crop and food security

Crop sensing using nano-enabled diagnostics technology can be dovetailed with autonomous machines monitoring crops at an individual plant level. As a result, they may provide best technological solutions to enhance active surveillance with more antemortem diagnostic information (Vo-Dinh et al. 2006). These devices contain one or more biosensors with nanosized components. If these miniaturized devices are implanted, they can be extremely useful in delivering real-time information about the health status of agricultural crops. For example, with the ability to detect proteins down to a few molecules, the field of diagnostics can be brought to the fundamental level of a single cell. Ruengruglikit et al. (2004) have developed an electronic tongue for inclusion in food packaging that consists of an array of nanosensors that are extremely sensitive to gases released by food as it spoils, causing the sensor strip to change colour as a result, giving a clear visible signal of whether the food is fresh or not. Zhao et al. (2004) developed an ultrasensitive immunoassay for in situ pathogen quantification in spiked ground beef samples using antibody-conjugated silica fluorescent nanoparticles (60 nm). Cheng et al. (2009) demonstrated rapid detection of E. coli in food using biofunctional magnetic nanoparticles (20 nm) in combination with adenosine triphosphate bioluminescence. At the University of Manitoba in Winnipeg, microelectronics and nanotechnology have been combined to develop a nano-sensor that can help farmers in the early detection of grain spoilage during storage (Neethirajan et al. 2009). The advantage of this sensor system is that thousands of nanoparticles can be placed on a single sensor to accurately detect the presence of insects or fungus inside stored grain bulk. Because of the miniaturization and low power requirement, the nanosensors can be deployed and distributed into the crevices of grain bulk, where the stored grain pests often hide (Neethirajan and Jayas 2011). In fact, these achievements introduce new chances for innovation in the agriculture and food industry at immense speed, but uncertainty and health concerns are also emerging, and therefore, need to be addressed critically using myconanotechnology.

Non-target effects of myconanoparticles

Myconanotechnology has a large scope of potential applications in agriculture, food and textile industry, however,



the adverse impact of myconanoparticles has rarely been studied. Although research on the adverse effects of nanoparticles on agriculture and allied sector is progressing rapidly, environmental fate of nanoparticles is still in its infancy. Nano-aluminum oxide (Al₂O₃) could inhibit root elongation of corn, cucumber, soybean, cabbage and carrot (Yang and Watts 2005), while ZnO nanoparticle was reported to be one of the most toxic nanoparticles that could terminate root growth of radish, rape, ryegrass, lettuce, maize and cucumber (Lin and Xing 2007). Lee et al. (2008) analysed toxicity and bioavailability of copper nanoparticles to *Phaseolus radiatus* and *Triticum aestivum* employing plant agar test as growth substrate for homogeneous exposure of nanoparticles. The growth rates of both plants were inhibited and as result of exposure to nanoparticles and the seedling lengths of tested species were negatively related to the exposure concentration of nanoparticles. Similar research was undertaken on the toxicology of nano-Al₂O₃, nano-SiO₂, nano-magnetite (Fe₃O₄) and nano-ZnO on Arabidopsis thaliana, with the results showing that nano-ZnO (400 mg) could inhibit germination (Lee et al. 2010). Benn and Westerhoff (2008) revealed that the silver can easily leak into waste water during washing, thus potentially disrupting beneficial bacteria used in waste-water treatment facilities, or endangering aquatic organisms in lakes and streams. Detrimental effects of silver nanoparticles on Raffaelea sp. causing mortality of oak trees was also investigated and studies showed harmful effects of silver nanoparticles on conidial germination (Kim et al. 2009). Hu et al. (2010) demonstrated that both TiO2 and ZnO nanoparticles exert harmful effects including oxidative stress, inhibition of the activity of cellulase, DNA damage and mitochondria damage, on earthworms (Eisenia fetida) when their levels are higher than 1.0 g/kg in soil. Although, improved nanoparticle delivery systems need to be developed for specifically targeting the infected tissues alone and therefore, needs more focused studies. Before unknowingly dumping a huge amount of nanomaterials into the environment, we need to investigate the solubility and degradability of engineered myconanoparticles in soils and waters, to establish baseline information on their safety, toxicity and persistence in soil and aquatic life. Development of novel myconanoparticles must be followed by the assessment of their potential risks on human life and environment, and possible remedial measures.

Research needs and future prospects

The essence of myconanotechnology as a suitable biosystem for the synthesis of different kinds of nanomaterials is well established with great potential and promise for advanced diagnostics, biosensors, precision farming and targeted smart delivery systems (Table 3). In the foresee-able future, following issues need to be addressed from the nanotechnology and mycology view point for sustainable and precision agriculture.

- Size and monodispersity are two important issues in the evaluation of mycogenic synthesis of nanoparticles. Therefore, effective control of the particle size and monodispersity must be extensively investigated by varying parameters like fungus type, growth stage, cultivation medium, synthesis conditions, pH, substrate concentrations, source compound of target nanoparticle, temperature, reaction time, and addition of nontarget ions.
- Mechanisms of nanoparticle synthesis using fungus have not been clearly and deeply elucidated. Thus, more elaborated studies are needed to outline the exact mechanisms of reaction and to identify the enzymes and proteins which involve in mycogenic nanoparticle production. Hence, establishment of low-cost recovery techniques to make the synthesis process commercially feasible also needs to be undertaken.
- The use of fungi as a means to develop natural nanofactories has the added advantage that downstream processing and handling of the biomass would be much simpler. At present, mycogenic synthesis of nanomaterials of varying composition are limited and confined to metal, some metal sulphide, and very few oxides. An extension of the procedures to enable reliable synthesis of nanocrystals of other oxides, nitrides, carbides, etc., could make microbial synthesis a commercially feasible proposition.
- Myconanotechnology has the potential to revolutionize the agricultural and food industry with new tools for the molecular treatment of diseases, rapid disease detection and enhancing plant ability to absorb nutrients. Therefore, development of smart sensors and smart delivery systems will be helpful to protect crops against insect pest and plant pathogens. However, it is not easy to adapt a technology developed for genetic transformations in plants. Effective means of myconanoparticles application should be identified, and their behaviour, movement and accumulation within the plants should be understood.
- Fungus mediated synthesis of metal nanoparticles provide a safe synthesis route with better control over morphology of nanoparticles. The interaction of plant cell with the nanoparticles results in modification of plant gene expression and associated biological pathways which ultimately affect plant growth and development. Therefore, experimental trials are needed to understand the nanotoxicity to plants, possible uptake



Table 3 Emerging challenges and future perspectives of myconanotechnology in agriculture

Challenge(s)	Promising nanomaterial(s)	Future agro-perspective(s)	References
Nano-based diagnostic kits	Quantum dots, nanowires, nano- bioluminescence spray, nanoarray	Enhancing the ability of plants to absorb nutrients, fight diseases and withstand environmental stresses	Vo-Dinh et al. (2006), Bakar et al. (2011), Mousavi and Rezaei (2011)
Precision farming	Nanocapsules, nanocarriers, nanosensors, quantum dots, nanoencapsulated biopesticide, mycoherbicide and biofungicide, nanofibrous mats, dendrimers, nanoclay, nanocomposites	Provide accurate information along with remote sensing tools for real time monitoring of soil conditions, environmental changes and diseases and plant health issues	Rickman et al. (2003), Owolade et al. (2008), DeRosa et al. (2010), Rai and Ingle (2012), Karimi et al. (2012)
Monitoring and targeted action	Nanospheres, liposomal nanovesicles, nanoshells, nanocapsules, nanostructured catalysts	Maximize the output and minimize inputs through better monitoring and targeted action	Yang et al. (2009), Goswami et al. (2010), Mousavi and Rezaei (2011)
		Bringing more areas under cultivation by nanotechnology enabled environmental monitoring and management including cost effective water management	
Nanotechnology enabled sensors and smart delivery systems	Nano-structured catalysts, double capsulized nano-particles, nanowires, protein-coated nanocantilever, nanosensors, nanobioformulations, nanotracers	Detection of plant or food pathogens, nutrient deficiencies and soil moisture Biosynthesis of nano-structured for increasing the efficiency of biopesticides with lower doses	Perea-de-Lugue and Rubiales (2009), Nair et al. (2010), Neethirajan and Jayas (2011), Rai and Ingle (2012)
		Management of resistancedvelopment against pesticides in insect pest and plant pathogens	
Reduction of environment pollution	Nanoclay, nanowires, zeolites, nanostructured catalysts, nanoemulsions	Reduction of agricultural waste to minimise environmental pollution	Lang (2003), Dhillon et al. (2012)
Smart packaging	Polymerised silver nanoparticles, nanocapsules, nanofibers, nanoemulsions, nano-bioluminescence spray, nanobarcodes, nanofibrous mats, nano identification tags, nano-coatings and nano-dirt repellent plastic bags	Ensure food safety and security, as well as technology applications which alert the customers and shopkeepers when a food is nearing the end of its shelf-life.	Zhao et al. (2004), Ruengruglikit et al. (2004), Cheng et al. (2009), Neethirajan and Jayas (2011), Sharon et al. (2010)

and translocation of nanoparticles by plants, and physical and chemical properties of myconanoparticles in rhizosphere and on root surfaces.

Briefly, myconanotechnology is still in its infancy and therefore, focused research, development, and funding could potentially redirect nanotech efforts to achieve millennium goal of food security.

Conclusion

Mycogenic synthesis of nanoparticles has attracted a great interest in recent years, although, most of mechanisms related to their synthesis have not been elucidated yet; it is supposed that fungi will take measures when the toxic ions are present in their growth environment for protection. Since the cell surface of fungal biomass usually appears negative charge, and the secretion of cells are sticky, the ions will be attracted or attached on the cells due to the

electrostatic interaction or secretion adhesion. The functional reducing agents, metabolites and enzymes released by fungi to convert the toxic ions into non-toxic matters may have a specific role in nanoparticle synthesis. In addition, it cannot rule out the possibility that the nanoparticles were formed due to the precipitation. The better control on particle size, shape and monodispersity of nanoparticle synthesis by fungi is still being sought. In terms of the results of related studies, it can be understood that variety of fungal strains, growth medium, and synthesis conditions are responsible for the size and monodispersity of nanoparticles. The mycological methods to produce nanoparticles are still in the developing stage. Extracellular methods are appropriate for entrapment and immobilization of nanomaterials on the desired support. Intracellular methods may be suitable for bioinorganic composite films. The strategy of utilization of enzymes secreted by the fungi for subsequent formation of nanoparticles in vitro opens up the new exciting possibility of biosynthesis of nanoparticles of predefined chemical



composition and developing a rational, eco-friendly fungal enzymes based large scale bioprocess for nanoparticle synthesis. With the recent progress and the ongoing efforts in improving nanomaterial(s) (QDs, nanowires, nanoemulsions, nanosensors, nanofibrous mats, nanobiopesticides) synthesis efficiency, exploring their applications in agro-industry and assessment of environmental risks associated with these particles, it is hopeful that the implementation of myconanotechnology strategies on a large scale and their commercial applications in agriculture and allied sectors will take place in the coming years.

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