MYCONANOTECHNOLOGY:
GREEN CHEMISTRY FOR SUSTAINABLE DEVELOPMENT

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Mycology: Current and Future Developments

(Volume 3)

Myconanotechnology: Green Chemistry for Sustainable Development

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CONTENTS

FOREWORD

Nanotechnology is a fast-growing field of science that involves the 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. The most commonly employed procedures are based on physical and chemical techniques therefore it is reasonable to develop alternative biological methods with an important ecological impact.

Fungal systems have attracted much attention in this regard, as they are eco-friendly, biodegradable, easy to culture, nontoxic, low-cost and scale up, with a high wall binding capacity, thus heralding great promise for their application in biotechnology and industry. Formation of nanoparticles employing fungi and their application in medicine, agriculture and other areas is known as myconanotechnology. Nowadays, this kind of green synthesized nanoparticles have attracted great attention due to their outstanding biocompatibility and eco-friendly nature leading to explore new commercial uses and benefits of fungi.

The present book is organised into two sections, A and B. Section A will update readers on several cutting-edge aspects of the synthesis and characterization of nanoparticles through the use of fungi; then section B will describe some applications of myconanotechnology for management of bacterial and fungal diseases, pest control, as well as for application in medicine and agriculture making this a fairly new and exciting area of research with considerable potential for further development.

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PREFACE

Mycology is the study of fungi which has become a valuable science in the last 100 years as it has wide applications in agriculture, food and beverages industry, biofuel production, biotechnology, medicine, *etc.* Recently nanotechnology has emerged as a potential candidate due to its immense applications in other fields of science such as chemistry, biology, physics, materials science, engineering and biomedicine. Nanotechnology is the art of science of manipulating matter at nano scale design (1-100 nm). Myconanotechnology is an interface between mycology and nanotechnology which can be defined as the green synthesis of nanoparticles using fungi which is recently gaining attention due to their cost-effective, sustainable, resource efficient, simplicity and ecofriendly nature. While the synthesis of nanoparticles through conventional chemical and physical methods requires high energy, they are toxic and expensive too. Myconanotechnology has a wide range of applications like in agriculture, biomedical, electronics, textiles, cell and molecular biology, nanodevices, and many more. In the proposed book entitled Myconanotechnology, the editors have included the recent advanced approaches for studying mycology for the benefit of humankind. The book will cover the history and scope of myconanotechnology, green synthesis of nanoparticles by using fungi, the mechanism involved in such biosynthesis, applications of green nanomaterials in agriculture, industries, cosmetics, wood industry, biofuel production, therapeutics, diagnostics, *etc.* This book will be immensely useful to researchers, scientists of fungal biology, teachers and students, microbiologists, nanotechnologists, technocrats, and to those who are interested in myconanotechnology. The present book is intended to provide both students and researchers with a broad background to some of the fastest developing areas in current myconanotechnology. The chapters in the book are loosely grouped into two sections (Synthesis of nanoparticles and their applications in environment, industry, medicine and agriculture) in order to reflect the wider 'customers' or context of the particular myconanotechnological areas or activities. Each chapter is contributed by internationally recognized researchers/scientists, so the reader is given up-to-date and detailed information about myconanotechnology and various applications of fungi. It is to be noted that the authors of individual chapters are solely responsible for any scientific queries. We are indebted to the scientists and active researchers who have contributed their research work in the form of chapters and made this compilation a unique collection of recent studies in the field of myconanotechnology.

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CHAPTER 1

Fungi and Nanotechnology: History and Scope

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Abstract: Nanotechnology is one of the most fascinating areas of research, it is the cutting-edge technology that has a great impact on various application fields. Nanoparticles have been under consideration due to their applicability in almost every field. There are many methods used for the synthesis of nanoparticles but biological methods have proved to be superior. Among various biological sources, microorganisms have gained attention recently. Bacterial nanoparticle syntheses from terrestrial as well as from marine habitats have been frequently studied as compared to fungal counterparts. Recently, Fungal Nanotechnology has received much attention as it has a big role to play in future as well. During the last decades, marine fungi have been observed to exhibit novel nanotechnological application potentialities. This chapter deals with the history and emergence of myconanotechnology, focusing on terrestrial as well as marine fungal resources. Fungal nanoproducts have noteworthy scope in diverse fields. This chapter also discusses the scope of myconanotechnology in future.

Keywords: Fungi, History, Marine, Nanotechnology, Scope, Terrestrial.

INTRODUCTION

Nanotechnology involves designing, synthesizing and characterizing application-oriented materials and devices' smallest functional organization of which at least in one dimension is on nanometer scale. Materials at this scale exhibit unique properties than their bulk counterparts [1].

Nanotechnology is increasingly being used in agriculture, medicine, environment, textiles and opto-electronics for over the last two decades due to the tunable properties of nanomaterials [2]. Specifically, metal nanoparticles have applicati-

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

ons in resistance of arthropods [3], high prevalence of antimicrobial agents in different microorganisms [4], antitumor therapy [5]. Apart from that, metal nanoparticles have also increased interests of researchers as an alternative class of agents with antiviral, larvicidal, antiprotozoal, acaricidal, *etc* [6 - 8].

Numerous methods of nanoparticles synthesis, such as chemical, biological and physical, have been explored. Mostly, chemical methods are used in the fabrication of nanoparticles because it facilitates large quantities production in relatively short time with a good control on the shape, size and distribution [9]. In chemical methods, the variety of sizes and shape could be adjusted by controlling the reaction conditions and concentration of reacting chemicals. But, most of these methods are employ toxic chemicals, produce hazardous waste and energy intensive in nature. Similarly, physical methods such as microwave-assisted synthesis, laser ablation, sputter deposition, *etc*., are available for synthesis of nanoparticles. Due to involvement of high temperature, radiations, pressures in physical methods biogenic synthesis of nanoparticles is gaining interest [10].

Investigation and development of optimized protocol for the synthesis of nanoparticles of tailored size and shape is required advancement of nanotechnology [11]. Hence, scientists around the globe are foreseeing biological systems that can be used as an efficient system for the synthesis of metal nanoparticles. These biological systems include microorganisms *i.e*. bacteria, cyanobacteria, fungi, algae, *etc*. and plants for intracellular and extracellular synthesis of nanoparticles [12 - 14]. Among these microorganisms, fungi are most commonly used due to their wide distribution in nature and hence, play an important role in synthesis of nanoparticles. In 2009, Mahendra Rai and his co-workers have proposed the term "Myconanotechnology" for integrated research on mycology and nanotechnology [15]. Since then in last one decade myconanotechnology is gaining interest of scientists, policymakers and entrepreneurs due to many advantages and revenue generations in pharmaceuticals, chemical and healthcare industries.

FUNGI AS AN EFFICIENT SYSTEM FOR SYNTHESIS OF NANOPARTICLES

Fungi have various enzymatic and protein machinery which can be used as reducing agents, subsequently used in synthesizing nanoparticles from their salts. Fungi secrete a large amount of protein at a very high rate and therefore, the conversion of salts to nanoparticles is at a very high rate compared to bacteria. Additionally, fungal biomass also grows faster compared to bacteria under the same conditions. Mycelia of fungi offer many folds larger surface area for interaction and facilitate synthesis at a higher rate compared to bacteria [16 - 19].

Mycelia of fungi can tolerate high flow pressure and agitation in bioreactors compared to other microbes and plants [20]. The cell wall of fungi gives mechanical strength to tolerate osmotic pressure and environmental stress [21]. Another advantage is economic viability of fungi as large-scale synthesis is possible with small amount of biomass [22, 23]. These characteristics make fungi an efficient system for the synthesis of nanoparticles.

HISTORY, OCCURRENCE OF FUNGI AND EMERGENCE OF MYCONANOTECHNLOGY

The fungi have ancient origin and evidence that indicate the fungi possibly initially appeared billion years ago, therefore, record from fossil is very little. This was confirmed by the fungal hyphae evidence within the from oldest plant fossils. At the present knowledge on fungi called *Prototaxites* from terrestrial plantlike fossil source were common in all parts of the world throughout in Devonian period (419.2 million to 358.9 million years ago). Filamentous fungi from fossil of *Tortotubus protuberans* till the date oldest fungal species on the globe from Silurian period (440 million years ago) from fossil of terrestrial source [24]. During 20th century, classification of fungi was done and in the middle of the 20th century fungi were solely recognized as a distinct kingdom. This is based on their mode of nutrition (using excretion of digestive enzymes and absorption of externally digested nutrients).

Fungi are ubiquitous and occur abundantly in **(a)** terrestrial as well as in **(b)** marine environment.

Terrestrial Systems and Various Sources of Fungi

A terrestrial ecosystem involves the interaction of biotic and abiotic components and land-based community of organism in specific area, *e.g* of the terrestrial ecosystem are tundra, taigas, temperate deciduous forests, tropical rainforests, grasslands, and deserts. The types of terrestrial ecosystem were dependent on major four parameters *i.e*. temperature range in the area, average annual precipitation received, type of the soil and the light intensity received in the area.

Fungi are one of the largest terrestrial and aquatic organisms which play various roles in ecological and environmental balance. Till date, approximately 1.5 million fungal species were recognized by scientific communities [25, 26]. In worldwide, a minimum of 712285 extant fungal species are found, out of which 600,000 fungal species are found associated with terrestrial plants [27]. The use of current cultivation techniques has led to10% of the total fungi have been cultured [28].

CHAPTER 2

Fungal Nanobionics - Principle, Advances and Applications

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Abstract: Nanotechnology is the science of research and development at the nanoscale (*i.e.* 10^{-9} m) at least in one dimension. The capability of nanotechnology is often known to revolve around nanoparticles. The core versatility of the nanoparticles is the fact that they exhibit more significant properties than that of bulk counterparts. Nanobionics is the structural and functional study of biological systems which serve as a model for the design and engineering of materials and machines at the cistron level. Fungi have emerged as important systems for the synthesis of nanoparticles due to the production of extracellular enzymes which can utilize heavy metal ions and produce nanoparticles, easy to isolate and subculture on synthetic media due to low nutritional requirements, high wall binding capacity, simpler biomass handling and extracellular synthesis of nanoparticle help in easy downstream processing. Fungi can produce nanoparticles both extracellularly as well as intracellularly. For example, the synthesis of silver nanoparticles has been reported utilizing many ubiquitous fungal species including *Trichoderma, Fusarium, Penicillium, Rhizoctonia, Pleurotus* and *Aspergillus.*

Extracellular synthesis has been shown by *Trichoderma viridae* while intracellular synthesis was shown to occur in a *Verticillium* species, and in *Neurospora crassa* whereas the synthesis of gold nanoparticles has been reported utilizing *Fusarium, Neurospora, Verticillium, yeasts,* and *Aspergillus*. In this chapter, we further focus on the applications of fungal nanobionics in various industries.

Keywords: Extracellular synthesis, Fungal nanotechnology, Nanobionics, Nanoparticles.

INTRODUCTION

Nanotechnology finds its applications in many fields like medicine, cosmetics, device development, *etc*. Nanotechnology relies on the development of nano-particles from various sources. Based on the source of the nanoparticles, they are classified mainly as organic and inorganic.

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

Organic/Carbon-based nanoparticles: Carbon-based nanoparticles are made up of two materials carbon nanotubes (CNTs), made up of graphene and fullerenes. CNTs are further classified into single-walled carbon nanotubes and (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs thermally conduct along the length and non-conductive across the tube and have higher strength. CNTs are being utilized as adsorbents for toxic chemicals from the manufacturing industry or pharmaceutical wastewater and microbial fuel cells [1].

Inorganic nanoparticles: Nanoparticles with magnetic, noble metal (*e.g.* palladium, gold and silver) and semiconductor nanoparticles (*e.g.* titanium dioxide and zinc oxide). Metal nanoparticles are produced from metal precursors with the help of various chemical, electrochemical, or photochemical methods. Metal nanoparticles can absorb small molecules and have high surface energies. These nanoparticles can be synthesized and modified with various chemical functional groups, which allow them to be linked with antibodies, ligands, and drugs of interest, resulting in opening a wide range of potential applications in biotechnology, magnetic separation, preconcentration of target analytes, targeted drug delivery and vehicles for gene and drug delivery [2]. Metal and metal oxide-based nano-adsorbents play a significant role in the removal of pollutants from wastewater, coating of magnetic nanoparticles with other supports resulting in an increased adsorption efficiency. For example, 6.16% higher by coating silver. Similarly, a Co_3O_4-SiO_2 magnetic nanoparticle coated with nylon 6 enables the adsorption of 666.67 mg/g Pb (II) from wastewater at 298K. The iron oxide nanoparticles implanted on hyperbranched polyglycerol were able to remove nickel, copper, and aluminium from wastewater in 35s successfully.

These nanoparticles can be prepared by different methods like physical and chemical methods. Physical methods used for the synthesis of nanoparticles are energy-intensive and expensive, while chemical methods are based on hazardous chemicals. Hence, there is a need to develop eco-friendly and biocompatible [3] techniques for nanoparticles synthesis. Biological synthesis of nanoparticles can be an alternative to physical/chemical methods of nanoparticle synthesis *i.e.* nanobionics. These methods provide rapid synthesis, better control over size and shape, less toxicity, cost benefits and are eco-friendly [4]. Plant, bacteria, yeast and fungi, can be utilized as effective producers of nanoparticles using principles of nanobionics. Thus, biomimetic nanoparticles formations are known to be much more 'cleaner' and 'greener', owing great relevance to the field of nano-biotechnology. The synthesized nanoparticles with a larger proportion of surface area, higher surface energy, spatial confinements and perfect dimensions are known to have potential benefits in the field based on catalytic, magnetic, electronic and optical properties. The realm of nanotechnology limits its boundaries with bio-based nanoparticles, which gives a promising benefit on protecting the environment through pollution prevention, treatment, and cleanup of contaminates, among others. Also, the broader spectrum of synthesized nanoparticles morphologies and considerably faster biosynthesis rate in cell-free filtrate (due to the higher number of proteins secreted in fungi) make this a specifically exciting route.

Fungi have evolved as an important system for the synthesis of nanoparticles due to their ability to produce extracellular enzymes which can utilize heavy metal ions and can effectively produce nanoparticles. It was easy to isolate and subculture on synthetic media due to low nutritional requirements, high wall binding capacity, simpler biomass handling and extracellular synthesis of nanoparticles which help in easy downstream processing [4], tolerance to high agitation and low pressure as compared to bacteria and plants [5]. There are two strategies for the synthesis of nanoparticles by fungi (1) top-down and (2) bottom-up. In the former approach, the process starts from bulk material and is scaled down to produce nanoparticles, while in the latter, from the small particles (atoms) nanoparticles are assembled.

Green Synthesis of Nanoparticles

Mechanisms of Mycosynthesis of Nanoparticles

Studies on the understanding of mechanisms of synthesis of nanoparticles are underway and a lot is to be deciphered. Briefly, the following mechanisms are proposed for mycosynthesis of nanoparticles. Fungi can produce nanoparticles both extracellularly as well as intracellularly. During intracellular synthesis, fungal biomass is treated with the metal ion solution and incubated in the dark for 24 hrs [3]. Heavy metal attaches to the fungal cell wall with the help of proteins or enzymes present on it *via* electrostatic interactions. Enzymes present in the cell wall reduce the metal ions, leading to aggregation of metal ions and formation of nanoparticles [5, 6]. Extracellular synthesis assumes the interaction of metal ions and releases enzyme mainly reductase with subsequent formation of nanoparticles in solution. Extracellular synthesis of nanoparticles has advantages as it does not require lysis of fungal cells, downstream processing for recovery and purification of nanoparticles [7] whereas, in the case of intracellular synthesis recovery and purification of nanoparticles from fungal biomass is a laborious task that requires analytical equipment and long processing techniques.

Various types of nanoparticles such as silver, gold, *etc*. have been successfully produced by various groups of fungal genera. For example, the synthesis of silver nanoparticles has been reported utilizing many ubiquitous fungal species including *Trichoderma, Fusarium, Penicillium, Rhizoctonia, Pleurotus and Aspergillus.* Extracellular synthesis has been produced by *Trichodermaviride*

CHAPTER 3

Green Synthesis of Fungal Mediated Silver Nanoparticles with Potential Biocontrol Application

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Abstract: Era of nanotechnology has played a significant role in various aspects of our life. These are minute particles having vast roles. Numerous techniques have been employed for its synthesis. Previously chemical and physical approaches were exploited for their synthesis but nowadays researchers are leaning on biological entities for their creation. And this terminology is green chemistry which does not harm the environment. Mycogenesis plays a terrific role in producing nanoparticles as they contain various enzymes and proteins playing a role in reducing the metal nanoparticles. Metal nanoparticles such as silver nanoparticles act as an efficient biocontrol agent. They were explored to control different types of pests, pathogens, microbes, *etc*. Different mechanisms were used for controlling the pathogens. They are effective due to broad-spectrum efficiency and ruin the cells by binding with phosphorus and sulphur present in the structure of protein and DNA. They are toxic to a wide range of microorganisms. This chapter focuses on the synthesis of silver nanoparticles using different fungal agents and the processes involved. Further, how the prepared particles have prospective application in the control of living organisms, description of all the pathogens against which silver nanoparticles were effective has been provided. This review will comprehensively provide some knowledge regarding the biocontrol application of myconanotechnology.

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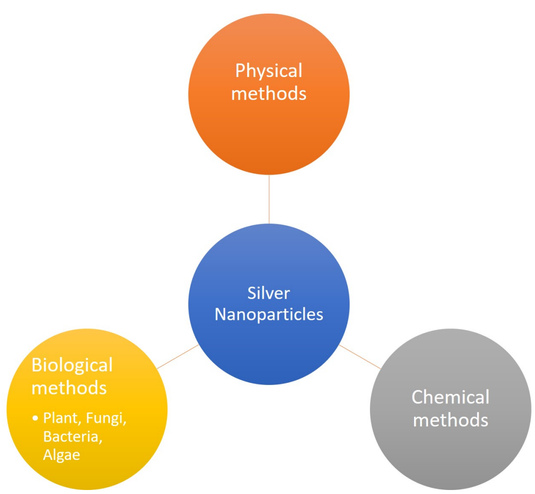
Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

Keywords: Biocontrol agents, Fungi, Nanoparticles, Silver.

INTRODUCTION

Nanoparticles made up of metals have appealed to researchers for two decades due to the unveiling of distinctive properties. Numerous chemical and physical procedures were employed to synthesize the desired kind of nanoparticles. Though these techniques were providing fruitful results but they involved hazardous chemicals. Reduction of extremity necessitated developing a technique benign towards our environment and minor toxicity. Here comes the green chemistry or the procedure involving the synthesis of nanoparticles using biological bodies (BB) which is also termed biogenic synthesis. Antimicrobial activity is of utmost importance while synthesizing nanoparticles using green chemistry and among metal nanoparticles, those made up of silver hugely act as antimicrobials. They possess both antibacterial and antifungal activity [1]. Silver nanoparticles have gained attraction towards its implications in various fields. Various bacteria [2, 3], algae [4, 5], fungi [6, 7], and plants [8, 9] were studied or used for silver nanoparticle synthesis. The involvement of BB in a procedure shows efficacious results and compassion towards the environment. Exploration has led to the expansion of nanotechnology, generating 'myconanotechnology'. In this area, the fungus is explored to obtain nanoparticles. Numerous researchers have reported the synthesis of various metal nanoparticles by a diverse range of fungi [7, 10 - 12]. Several pragmatic and beneficial aspects can be achieved using fungus as a source of nanoparticles than other microbial agents. One such advantage is the simplicity of the scale-up process making the whole process a lucrative one [13]. Fig. (**1**) shows the schematic diagram summarizing the topics of this chapter.



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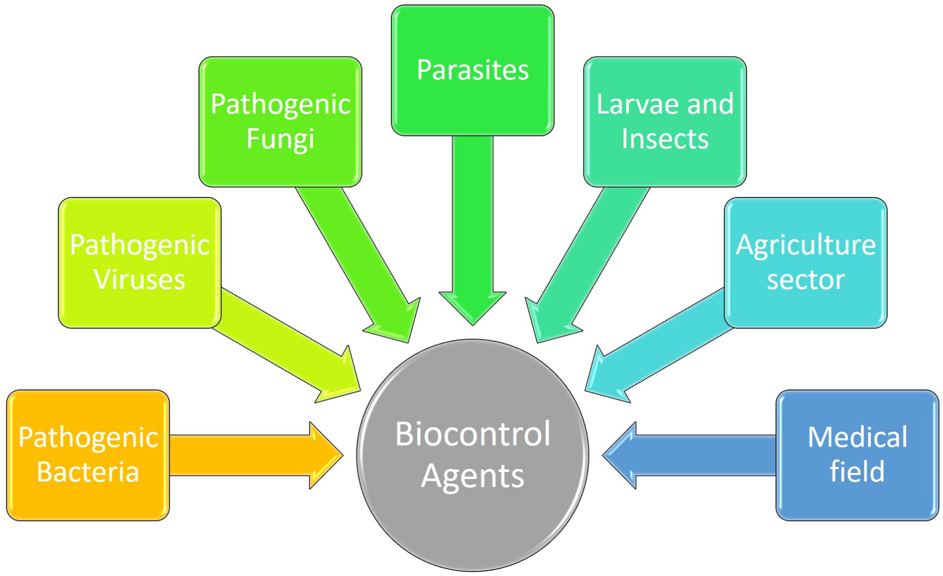

Fig. (1a and b). Schematic diagram of the silver nanoparticles synthesis and their application as biocontrol agents.

Nowadays, it has been reported and observed that the chemical agents used against the harmful pests in the agriculture sector have reported severe ill effects on living beings and our environment. The agricultural sector has used nanotechnology for various aspects. It has been reported that nanoparticles can play a vital role as pesticides, plant growth regulators, and in the process of fertilization when produced using green chemistry [14]. This technology helps in the augmentation of crop productivity by providing novel agrochemicals and efficient ways to deliver. The promising role alleviates the hope of reduced usage of chemical agents. There are groups of researchers inclined towards the synthesis of eco-friendly pesticides and they are herbicides and nanoparticles-based pesticides. They are exploring them for their antimicrobial activity to safeguard the crops against infections. For the exclusion of chemical-based pesticides in farming, a widespread study on nanoparticle-based systems is obligatory [15]. There are reports that nanosilver is effective against phytopathogen *Colletotrichum gloeosporioides* [16]. *Trichoderma longibrachiatum* has been explored to prepare silver nanoparticles and further, the prepared nanoparticles were used against nine phytopathogenic fungi showing effective results [17].

Not only agriculture, even the medical field saw the expansion of nanotechnology applications. Various infections caused by microbial agents have become resistant to antimicrobial treatment available so biogenic silver nanoparticles gained pervasive usage due to their broad-spectrum antimicrobial properties, safety, and

CHAPTER 4

Microbial Mediated Synthesis, Characterisation and Application of Selenium Nanoparticles

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Abstract: The development in nanotechnology, specifically the nanoparticulate system, has a great impact on medicine, engineering and other scientific areas. Inorganic nanoparticles such as silver, gold, zinc oxide, selenium, iron, lead, platinum and copper, *etc*. were found to exhibit antimicrobial, antioxidant and other biological activities, used as biosensors and also used in different fields of engineering. In the 21st century, microorganisms and plant parts are playing a major role in the synthesis of inorganic nanoparticles. Green synthesis of inorganic nanoparticles becomes preferable to other approaches because of its eco-friendly and non-toxic approach. Additionally, the active molecules of plants (Tannins, flavonoids, terpenoids, saponins, proteins and glycosides) which act as capping and reducing agents in the synthesis of metal nanoparticles could make them most suitable for biomedical applications. This green approach fascinated researchers across the globe to explore the potential of different microorganisms and plants in the synthesis of inorganic nanoparticles. Selenium nanoparticles are one of the inorganic nanoparticles which are widely used in the area of medicine and engineering. In this chapter, we discussed the green synthesis using microorganism and Agri based products, characterisation and various applications of selenium nanoparticles.

Keywords: Applications, Characterisation, Green synthesis, Nanoparticles, Selenium.

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

INTRODUCTION

Nanoparticles

In the 21st century, nanotechnology is known as an emerging interdisciplinary field, involving biology, physics, medicine, chemistry and material science. Nano is derived from the Greek word 'Nanos' that refers to things of one-billionth (10^{-9}) in size. Richard Feynman is the one who has presented the primary concept of nanotechnology in his lecture topic entitled "There's plenty of room at the bottom" at the American Institute of Technology in 1959. Nanoparticles have typical physical, chemical, magnetic, electronic, mechanical, thermal, optical and biological properties than bulk materials. This is due to the large number of surface atoms, large surface energy, spatial confinement and reduced imperfections in NPs because of decreasing the dimension of particles to the nanoscale.

Nanoparticles are usually 0.1 to 100 nm in each spatial dimension. Hence, nanoparticles are considered building blocks for the next generation of optoelectronic devices and chemical and biochemical sensors [1, 2]. Monodispersed nanoparticles with particular shapes have wide applications in the areas of optics, computation, medical diagnostics, cancer therapy and drug delivery. Therefore, interest in developing monodispersed nanoparticles with different sizes and shapes has increased in the past decade.

Conventionally, nanoparticles were synthesised *via* physical, chemical, and biological methods. There are two approaches, which play very significant roles in nanoscience and nanotechnology, for the synthesis of nanomaterials, namely the "top-down" and the "bottom-up" methods (Fig. **1**). The top-down approach is mainly used by physicists, and the bottom-up approach is used by chemists. There are advantages and disadvantages to both approaches. In the bottom-up approach, the nanostructured building blocks are synthesised and then assembled into the final structures, *i.e.* atom by atom, molecule by molecule or cluster by cluster, often by self-assembly. The colloidal dispersion is an example of a bottom-up approach in the synthesis of nanoparticles. The bottom-up method is suited for generating uniform particles of distinct size, shape, and structure.The major challenge in the bottom-up approach is the problem of stability because in most cases, the particles are formed in a solvent medium and thus have a better chance to collidewith each other and undergo aggregation [3, 4].

The top-down approach begins with suitable bulk material being broken down into particles of nano dimension by various physical processes like casting, moulding, rolling, forging, extruding, machining, etching, *etc*. During these operations, there is no control over the positions of individual atoms. This results in defects and impurities and is not suited for the synthesizing monodispersed particles. The top-down techniques are most apparent in the computer industry in the fabrication of microprocessors by lithography [5].

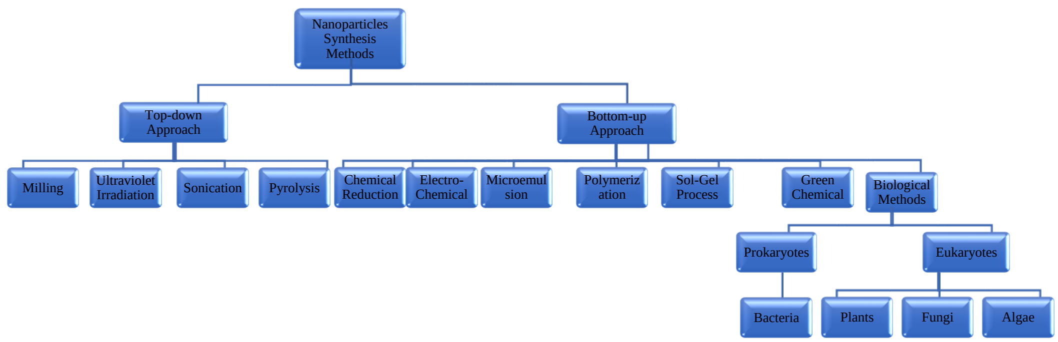

Fig. (1). Top-down and Bottom-up approaches in nanoparticle synthesis.

Various studies indicated that the size, shape, stability, as well as physical and chemical properties of the metal nanoparticles, are largely influenced by the experimental conditions such as the kinetics of interaction of metal ions with reducing agents, and interaction of the stabilising agent with metal nanoparticles [6 - 8]. Hence, the design of a synthesis method in which the size, morphology, stability and properties can be controlled has become a significant field of interest.

Selenium

Selenium (Se) is a non-metal and it has strong antagonistic biological interactions with several toxic metals, like cadmium (Cd), mercury (Hg) and silver (Ag) and showing less effectiveness against the toxic effects of lead (Pb). Less effect over Pb because PbSe is more easily oxidised compared to other metal selenium compounds. Hence, Se could be used as protection against the toxic effects of these metals [9, 10]. Underwood studied the effect of Se against chalcophile toxic metals, such as Hg and Cd and reported that the high intake levels of Se have a strong protective effect against the toxicity [11]. Clinical trials from the United States stated that Se supplementation had been shown to protect against prostate, colorectal, lung and liver cancer [12]. Nevertheless, a high intake of Se is needed for optimal protection against HIV disease, some forms of cancer and cardiovascular diseases than the intake needed for prevention of manifest Se deficiency diseases [13, 14]. Human daily intake of Se should not be more than 400 µg. Se has the transitional properties of both metal and non-metal, and is also available in the organic and inorganic state. The organic forms of Se are selenocysteine, selenomethionine, and the inorganic forms are selenite, selenide

CHAPTER 5

Green Synthesis of Nanoparticles using Fungal Extracts

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Abstract: Nanotechnology involves the synthesis of nanoparticles (NPs) and paved the way for the possibility of applications in different fields such as pharmaceutical science, industry, environment and biosensor technology. The metal nanoparticles synthesis using fungal extract is gaining momentum due to their novel chemical, optical, electrical, and magnetic properties. The mycelial biomass is found to be more resistant against pH, temperature, agitation and pressure compared to bacterial and plant extract and thus more appropriate for industrial production. The nano-sized particles synthesized by green chemistry are of better quality than the ones made by chemical reduction methods such as laser ablation, metallic wire explosion, photochemical or radiation reduction and sonochemical method. The chemical methods can pose a risk to environmental and animal health due to release of the hazardous toxic component. Therefore, nanoparticles synthesis using fungal extract could be an ecofriendly alternative to chemical-based methods as green synthesis has the lesser possibility of such component release. The fungal extract comprises a plethora of secreted extracellular proteins, enzymes, vitamins and ions which are responsible for the reduction and stability of nano-size metallic particles. The biogenic nanoparticles thus produced have attained much interest due to their composition, shape and size, photochemical, optical and chemical properties. The nanomaterials have applications in various fields such as biosensor technology, DNA based techniques, metabolomics, antimicrobial agents, cancer cell treatment, protein engineering, purification of water and degradation of pesticides, synthetic biology, downstream processing and delivery of therapeutic compounds.

Keywords: Biogenic synthesis, FTIR, Fungal Extract, Fungi, Metal nanoparticles, TEM, XRD.

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

INTRODUCTION

The concept of nanotechnology, though considered to be a modern science, has its history dating as back as the 9th century. Nanotechnology involves the production of metal nanoparticles (NPs), nowadays is a vastly studied area because of their photoelectrochemical, chemical, optical and electronic properties, particularly in noble metals such as silver, gold and copper [1]. Nanoparticles of gold and silver were used by the artisans of Mesopotamia to generate a glittering effect on pots. The foremost scientific description of nanoparticles properties was reported in 1857 by Michael Faraday in his famous article "Experimental relations of gold (and other metals) to light" [2]. The revolution of nano work conceptually started in the early 1980s, with the first paper on nanotechnology being published in 1981 by K. Eric Drexler of Space Systems Laboratory, Massachusetts Institute of Technology [3]. Nanoparticles are particulate dispersions or solid particles with a size in the range of 10-1000nm. Nanotechnology is being utilized in offering many new developments in the fields of biosensors, biomedicine, bionanotechnology diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders [4].

Recently there are various biological approaches of using fungal, plant or bacterial extract to reduce precursor metallic ions to elemental state. Fungal species specifically are known to be widely used as a promoter for the synthesis of nanoparticles because of high biomass, the large amount of enzyme production and easy handling. Their capability of transforming or degrading complex hazardous environmental compounds renders them, excellent bioremediation agents. The NPs synthesized by biological materials possess various unique properties and thus have many advantages over other methods that produce different shapes, sizes and voids. This chapter is mainly devoted to the preparation of various nanoparticles using fungi as biological materials and their applications.

APPROACHES FOR SYNTHESIS OF METAL NANOPARTICLES

The development of reliable/suitable processes for the synthesis of nanoparticles is an utmost aspect of current nanotechnology research. Both physical and chemical methods have been commonly used for various types of nanoparticles synthesis with particular sizes and shapes (Fig. **1**). Despite the high efficiency of chemical methods in producing NPs, they generally release toxic and hazardous pollutants in the environment during NPs synthesis process. Besides these chemical methods, the bottom-up or top-down techniques are generally used for NPs synthesis. The former approach entails mechanical breakdown or grinding of the larger piece of metallic material to mini scale particles, whereas the latter approach comprises the biological or chemical mediated synthesis of the nanostructure. The above-mentioned processes cause controlled condensation of solute molecules that formed NPs of desired shape and size. The following are the examples of commonly used methods for nanomaterial synthesis such as thermal synthesis, sputtering, Inert gas condensation, lithography, laser ablation, aerosol, radiolysis, reduction, sol gel technique, *etc*.

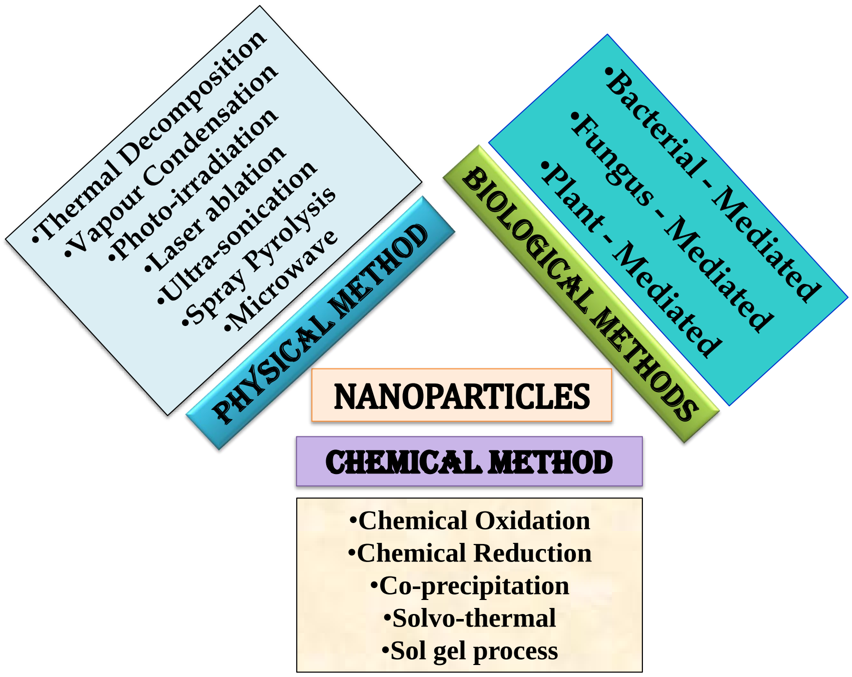

Fig. (1). Different methods for NPs synthesis.

Evaporation-Condensation

Various kinds of metal nanomaterials such as Ag, Au, PbS, WO_3, SnO_2, ZnO and fullerene, have previously been produced using the evaporation/condensation method [5, 6]. The technique involves the organic or inorganic material evaporated at a very high temperature usually in the furnace and the gaseous phase would be converted into homogenous nucleation by coalescence of atoms. The vapour pressure and pressure of the gas will determine the size and stability of NPs [7]. This method of synthesis of various kinds of NPs has several shortcomings, such as the requirement of large space for adjustment of the furnace, requiring more consumption of electricity (~10 kilowatts) for preheating for a long time to attain a stable operating temperature. Apart from this, the agglomeration of NPs is the major problem faced by the method [8].

CHAPTER 6

Extracellular Biosynthesis of Gold and Silver Nanoparticles using Fungal Extracts

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Abstract: Nanoscience has opened new vistas to manage phytopathogens, improve crop productivity by the development of new varieties, and control infectious diseases in humans. Silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) are highly acclaimed for their wide potential application in various fields. Chemical and physical methods of synthesis of AgNPs and AuNPs are widely used; however, such methods possess numerous setbacks, such as the production of toxic residues and indispensable need for high energy. Biosynthesis of nanoparticles is a cost-effective and environmentally friendly method. A plethora of species of plant, bacteria, fungi, *etc.* is available with potential biosynthesis ability. Fungi are a highly preferred organism owing to the ability to secrete a large number of extracellular enzymes, metal toxicity tolerance and bioaccumulation ability, and ease of handling of its biomass. Extracellular enzymes act both as reducing as well as capping agents. Two different methods are used by fungi for synthesis *viz.,* intercellular and extracellular synthesis. Extracellular synthesis is preferred over intercellular as it bypasses several down streaming processes. During the reduction process, the metal ions (Ag^{2+} and Au^{3+}) are converted to an elemental state (Ag^0 and Au^0) which is in the nano range. Due to their large surface-to-volume ratio and other properties, they become very effective against other pathogens. There is an excellent prospect of the use of nanoparticles in the field of agriculture and health and nanoparticles synthesized using a biological method involving fungi could be a boon.

Keywords: Bacteria, Biosynthesis, Extracellular synthesis, Fungi, Nanoparticles, Nanoscience, Phytopathogens.

INTRODUCTION

Since time immemorial, chemicals have been utilized to manage the phytopathogens directly or indirectly, and perhaps the use of chemical pesticides

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

is inevitable, taking into consideration the incessant increase of the human population. Unfortunately, pesticides have numerous setbacks which bring them to the limelight of criticism. Such conventional methods adversely affect the non-target organism and environment as a whole. Although systemic fungicides are known to have potential controlling effects, nevertheless, indiscriminate use of systemic fungicides leads to the development of resistance due to its specific site of action. Detrimental and inimical effects of pesticides can be attributed to their high toxicity, non-biodegradable nature, and residual activity. Therefore, there is an utmost need for an alternate avenue to combat the phytopathogens, and employing nanotechnology in agriculture could be a boon. Such one application is the use of nanoparticles (NPs) as nano-fungicides to manage phytopathogens. A nanoparticle by virtue of its high surface-to-volume ratio has high efficacy and durability and also delays the development of resistance in pathogens. Several metallic nanoparticles such as gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), copper nanoparticles (CuNPs), Platinum nanoparticles (PtNPs), Zinc nanoparticles (ZnNPs), *etc*. have been synthesized that can be exploited astoundingly to manage plant Pathogens. Three important methods are widely used for the synthesis of metallic nanoparticles (AuNPs & AgNPs) *viz.,* chemical method, physical method, and biological method. Physical and chemical methods are capable of generating large quantities of NPs with a defined shape and size in a short duration but these methods also have some major setbacks such as complex and out of date protocol requiring high expenditure, inefficient and produce hazardous toxic wastes that are inimical to the environment as well as to the human beings [1]. Propitiously "*Biosynthesis method*" satisfies almost all the criteria to fit and is an eco-friendly and efficient method for the synthesis of nanoparticles. Biological methods of synthesis are carried out by using plant extract, fungi, bacteria, or their metabolites which act both as reducing as well as stabilizing agents [2]. Biosynthesis of metallic nanoparticles using microorganisms is attracting the interest of many, which is a blooming environmentally safe science of nanoparticles synthesis with well-defined sizes, shapes, and controlled mono-dispersity [3].

The Silver nanoparticles (AgNPs) are one of the widely studied and utilized nanoparticles. It is known to have remarkable inhibitory/antimicrobial effects against various plant pathogens by virtue of its high surface area to the volume ratio [4]. Silver (Ag) is the second-most malleable and ductile element after gold (Au). Just an ounce of silver can be drawn into 8,000 feet long wire. The silver was first mined in Anatolia (present Turkey) between 5,000 and 6,000 years ago. Around 700 B.C., early Mediterranean civilizations started using silver as currency. The silver has powerful antibacterial/antimicrobial properties, which have been well known to humans during the times of the ancient Phoenicians, who kept their wine and food items fresh, by keeping them into silver vessels.

Nowadays, silver is widely used in health care tools such as bandages, surgical instruments, stethoscopes, *etc.* Unlike other antibiotics, silver also prevents bacteria from developing resistance to it.

Silver now has been accepted as a replacement for agrochemicals and has the potential to manage microorganisms. It has been used as a foliar spray to stop fungi, moulds, rot, and many other plant diseases. Silver has also been reported to act as a potential plant growth stimulator [5].

Lately, gold nanoparticles (AuNPs) have attracted the interest of many due to their significant applications and astounding properties. AuNPs are important as drug carriers to treat cancer, heart diseases, *etc*. by virtue of their large bioavailable surface area, which confers effortless functionalization [6]. AuNPs are also found to have antimicrobial activity. It is used to remove environmental pollutants. AuNPs are also used as nanosensors to detect viruses and other pathogens [7]. Moreover, AuNPs are recalcitrant to chemical oxidation and are capable to remain stable in varied environmental conditions [8, 9].

Biosynthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) are highly acclaimed nanoparticles for their broad-spectrum antimicrobial properties [10, 11]. AgNPs are widely synthesized by using the chemical method and physical methods. In chemical methods, chemical reagents are used which act as the reducing agent and stabilizing agent. However, reagents such as sodium borohydride, potassium bitartrate, hydrazine, *etc*. are highly toxic and can lead to health hazards and various environmental consequences [1, 12]. Physical methods are also capable of generating large quantities of NPs with a defined size and shape in a relatively short period of time, but this method has some major setbacks which cannot be ignored, such as they are complicated, outdated, costly (as it requires energy in form of heat and pressure), therefore pose a major threat to the environment [1].

Biosynthesis of AgNPs provides advancement over chemical and physical methods as it is cost-effective, environment friendly, easily scaled up for large-scale synthesis, and importantly it doesn’t involve high energy or any toxic chemicals [13]. The biosynthesis method generates nanoparticles of defined sizes, shapes and controlled mono-dispersity [3]. Several microorganisms are standing as impeccable candidates for the synthesis of NPs. Among numerous microorganisms, fungi are one of the highly preferred organisms by virtue of their high tolerance and bioaccumulation ability towards metals and are excellent efficient secretors of enzymes. Other advantages attributed to the biosynthesis of NPs are economic viability and ease of biomass handling [14]. Furthermore, it requires simple nutrients, possesses high wall binding capacity, and also has

CHAPTER 7

Role of Fungal Nanotechnology in Bioremediation of Heavy Metals

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Abstract: The main sources of soil contamination are anthropogenic activities, which result in the accumulation of contaminants that can reach levels considered toxic. One of the main soils contaminants these days is heavy metals. These metals are bioaccumulative and are not biodegradable, so many of them are toxic when they exceed certain limits. Heavy metals, when accumulated in the tissues of plants, animals and humans, induce severe symptoms that can even cause death. Bioremediation is a widely used technology to decrease the levels of these metals in soils and waters using microorganisms, among which fungi stand out. Nanotechnology currently applies the bases of bioremediation using fungi at the nanoparticle level to treat soils contaminated with heavy metals. This chapter will discuss novel aspects related to heavy metals in modern agriculture, bioremediation and nanotechnology using fungi with bioremediation purposes.

Keywords: Agriculture, Fungi, Heavy metals, Nanotechnology, Soil contamination.

INTRODUCTION

Intensification in agriculture combined with industrialization, wars and mining have left a legacy of contaminated soil throughout the world [1, 2]. Since the urbanization began, the soil has been used as a solid and liquid waste dump beca-

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

use it was thought that once buried, over time, the pollutants would not represent any risk to human health or the environment and, somehow, they would disappear [3]. The main sources of soil contamination are anthropogenic, which results in the accumulation of contaminants that can reach levels considered toxic [4].

Heavy metals are a group of metals and metalloids that have an atomic density above 4 g cm^3. This group include toxic plant metals such as lead (Pb), mercury (Hg), arsenic (As), cobalt (Co), cadmium (Cd), vanadium(V), chromium (Cr), nickel (Ni), selenium (Se) and some others that are essential to plants, like manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu). These metals can affect animals, plants, and humans due to a long-term exposure [5].

In the actual agricultural soil context, bioremediation is a useful alternative to reduce the great accumulation of heavy metals [6]. This technique is an efficient, cost-effective and environmentally friendly strategy that uses microorganisms such as yeast, bacteria, algae and fungi to diminish heavy metal contamination [7]. At present, the use of microorganisms, particularly fungi, for the removal of heavy metals has been extensively investigated [8, 9].

Conventional technologies to decrease soil contamination are restricted by rigorous health policies and emergent contaminants. Fungi-based nanotechnology is an effective technology to treat soil contaminated with heavy metals. Nanotechnology studies materials and applications occurring at a very small scale. The technology use structures sizing less than 100 nanometers (nm) and includes developing materials or devices within that size [10].

Nanoparticles (NPs) have several special properties that include bulk material and also have exceptional visible properties, because they are small enough to confine their electrons and produce quantum effects [11].

The aim of this chapter is to discuss and present novel aspects about agricultural soil contaminated with heavy metals and the application of fungal nanotechnology to diminish the damage of these metals to the health of plants, animal and humans.

HEAVY METALS IN MODERN AGRICULTURE

The contamination of soils with heavy metals is a great problem around the world [12], created principally by human activities [13] such as agriculture, industrialization, technology, urbanization, and mining [12, 14] (Fig. **1**). Mineral processing and mining are considered the main sources of soil contamination [12, 15]. The contamination of soils with heavy metals could generate serious problems for plants, animals, human health and the normal balance of the ecosystems [16].

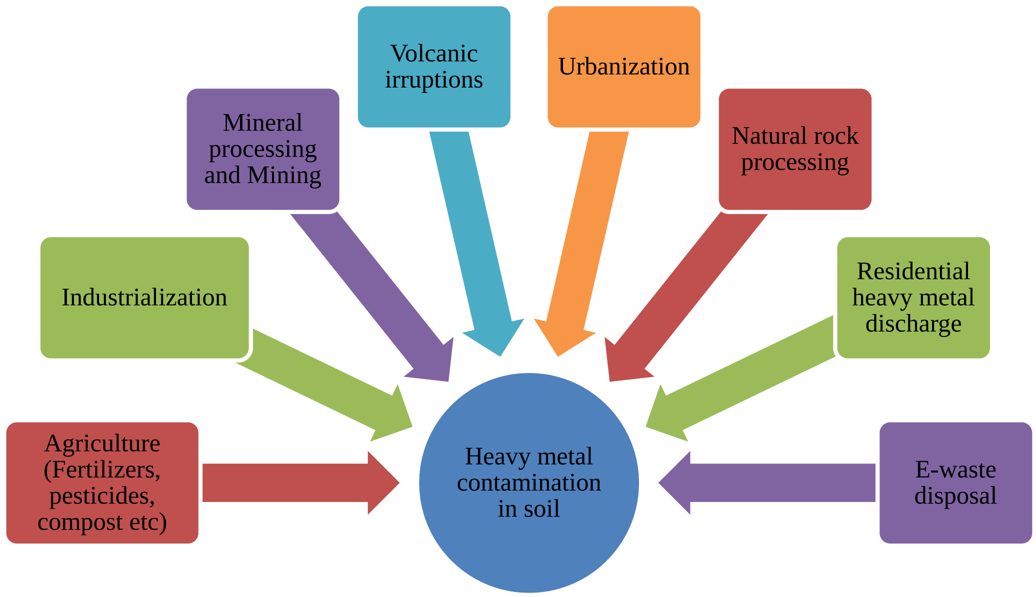

Fig. (1). Sources of heavy metals contamination in soil.

Several metals (Mn, Co, Zn and Cu) in low concentrations are essential for some metabolic functions in humans [17], but others do not have any positive effects on human health and they are considered carcinogens, such as As, Cr, Cd [17, 18]. Hg can produce ataxia in adults; in children, it affects language abilities as well as causes attention deficit [19], whereas Pb is considered possibly carcinogenic [18].

Agricultural production systems are one of the major sources of heavy metals because these metals can accumulate in the soil and be transferred to plants, entering the consumer chain, mostly in places where these processes are carried out intensively and without rest periods or rotation of crops [20].

According to Alloway [21], some heavy metals are directly related to pesticides (As, Cu, Mn, Pb, Hg, Zn), manure (Zn, Cu, As), fertilizers (Pb, Cr, Cd, Mo, Zn) and compost derived from waste conventional solids (Zn, Cu, Cd, Ni, Pb). Also, Laegreid *et al* [22]. state that phosphorous fertilizers are a Cd source due to apatite contents, which in addition to phosphorus (P) contains Cd in concentrations between 8 and 500 mg kg^{-1}. Similarly, the irrigation waters from treatment systems or contaminated sources increase the metal contents in the soil.

CHAPTER 8

Plant Fungal Disease Management by Nanotechnology

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Abstract: With the enormous increase in global population, there is an increasing number of individuals to feed. Crop loss has become the biggest issue worldwide. Insects (14%), weeds (13%) and various plant diseases (13%) play a very important role in crop losses. The loss caused by plant diseases single-handedly causes an estimated loss of 2 trillion dollars per year. Due to the increasing demand of food, the use of synthetic chemicals has become today's fastest, easiest and cheapest way to control loss causing agents. But due to the immense use of these chemicals, it induces adverse effect on the environment, human beings, animals and also depleting natural resources. In the current scenario, there is a need to introduce control measures which are effective and increase crop production but on the other hand, they must be less harmful for the ecosystem. After the introduction of irrational use of fungicides, there is always a posed threat to the living system, killing not only the target fungi but also affecting beneficial living systems. Besides, there is an increase in resistance against fungicides in the fungal pathogen. It is becoming necessary to reassess our strategies and achieve disease management by alternate approaches such as nanotechnology. Nanofungicides based on metals like silver (Ag), copper (Cu), *etc*. and nano-emulsion has been becoming an important technology to tackle fungal pathogen problems in agriculture, having immense potential to cope with the fungal pathogen in the future. However, very little work has been done to bring this technology to field level. Nanotechnology has substantially advanced in medicine and pharmacology, but has received comparatively less interest for agricultural applications. They aim at acting directly into the plant's part where the pest or disease attacks, which means that only the required amount of chemical is delivered to the plant tissue as medication. Nanoparticles may act upon pathogens in a way similar to chemical pesticides or the nanomaterials can be used as a carrier of active ingredients of pesticides, host defense inducing chemicals, *etc*., to the target pathogens. It is a more appropriate and suitable solution for crop protection and is also safer for the environment. It will improve agricultural output in the coming years by solving the above-mentioned problems in crop production therefore, extensive research work is needed. Nanotechnology

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may bring an evolution in industry as well as in the field of dealing with fungal pathogens.

Keywords: Agriculture, Delivery systems, Fungicides, Myconanotechnology nanodiagnostics, Nanofungicides, Nanomaterials.

INTRODUCTION

With the climate change scenario, the plants are becoming more venerable to many diseases. Among all the pathogen causing plant diseases, fungi are an important group of organisms that cause approximately $45 billion loss in crops all over the world every year. The demand for food is increasing day by day putting pressure on agriculturists for increasing the food supply. Fungi not only affect the seedling emergence by attacking the seeds but are also able to attack the plant tissues at different growth stages and lowering the yield ultimately [1]. Most of the disease management techniques involve the use of chemicals which we can achieve the necessary yield but these practices cost ecological damage. In spite of many advantages, like high availability, fast action, and reliability, a large portion of these chemicals does not reach their target and ultimately contaminate the ecosystem. Applied fungicides can be lost (90%) as a result of overflow and enter in the ecosystem disturbing both the agriculture and environment during its application in the open field. Therefore, farmers need to apply these fungicides in huge amount, and it becomes costly for them. In this situation, there is an increased motivation to develop cost-efficient, high-performing pesticides that are less harmful to the environment. Nanotechnology comes to focus, as it is the better way to manage plant diseases. Innovative tools are provided by nanotechnology to deliver agrochemicals at targeted area safely without disturbing the ecosystem. It has developed such carrier systems that cause the controlled release of compounds when needed; that is how the concentration of agrochemicals in the environment can be reduced [2, 3].

WHAT IS NANOTECHNOLOGY?

Nanotechnology is an advanced field of science, deals with production, manipulation and implication of matter at nanoscale. The concept of nanotechnology was seeded at first by a physicist "Richard Phillips Feynman" in 1959 and the term "nanotechnology" was coined by Professor Norio Taniguchi in 1969. The name is based on a Greek letter "nano" meaning dwarf. It is one billionth part of a meter or 10^{-9} m [4, 5]. Nanomaterials are very minute structures which range from 0.1 to 100 nm. Properties of these nanomaterials such as electrical conductance, magnetism, chemical reactivity, optical effects and physical strength vary from bulk materials due to their smaller size [6].

Nanomaterials are classified on the basis of dimension and on the basis of structural configuration. On dimensional basis nanomaterial are divided into three groups [7] and includes one, two and three dimensional nanoparticles.

Nanomaterials having less than 100 nm size with one dimension are grouped into one-dimensional nanoparticles, these are used for build-up of various chemical and biological sensors, solar cells, IT systems and optical devices. Nanomaterials having size less than 100 nm along two dimensions at least are grouped under two dimensional nanoparticles *e.g*. carbon nanotubes, fibers and platelets. Metallic nanomaterials having <100 nm in all dimensions *i.e*. quantum dots, dendrimers and hollow spheres, are three dimensional nanoparticles. On the basis of structural configuration, nanomaterials are grouped as Metallic nanoparticles, Nanocrystals Quantum dots, Carbon Nanotubes, Liposome, Dendrimer, Polymeric micelles and Polymeric nanoparticles [8, 9]. Two approaches *viz*., Top to bottom approach and Bottom to top approach [10] are used to synthesize nanoparticles (Fig. **1**).

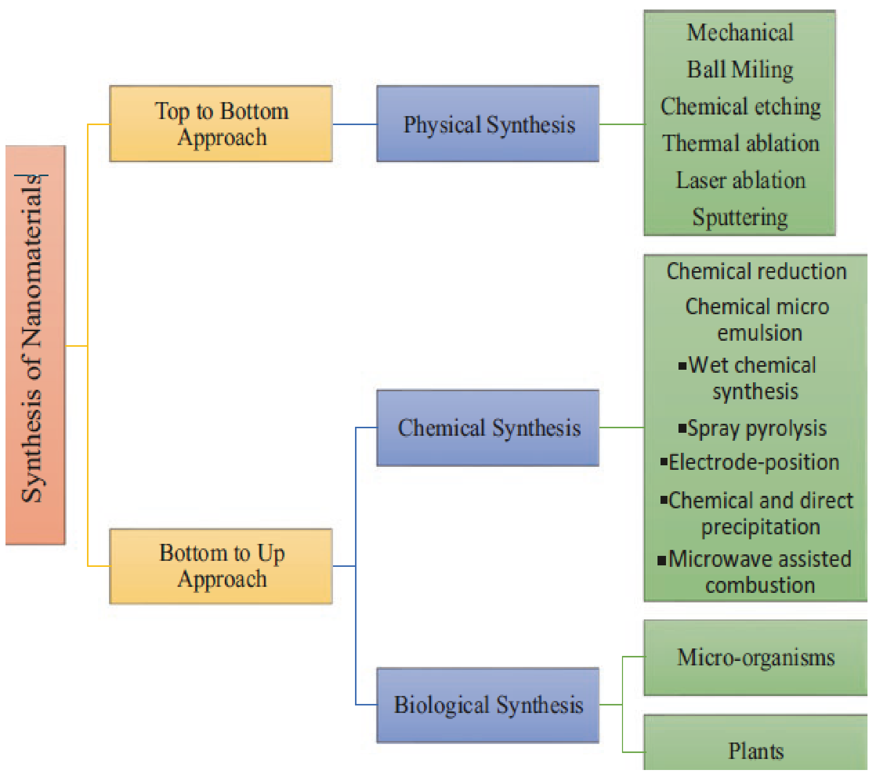

Fig. (1). Methods of synthesis of nanomaterials [10, 14, 15].

CHAPTER 9

In vitro Antifungal Efficacy of Nanomaterials against Plant Pathogenic Fungi and Oomycetes

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Abstract: Nanoparticulate (NP) substances have widely documented antimicrobial properties, yet their utilisation in the biocides and pesticides industries has yet to be fully exploited. This is particularly so in the pesticides industry, where their potential has not yet been realised. This mini review identifies the emerging trends identified in research characterising the *in vitro* antimicrobial properties of NP substances against fungal and oomycete phytopathogens. Nanoparticulate substances for which there was a sufficient depth of published studies on activity against fungal and oomycete phytopathogens are covered in this review, these include chitosan, copper, magnesium, silver and zinc. All substances displayed significant activity against a range of phytopathogens, though silver and copper-based NPs appear to be the most potent at relativity low (<50 ppm) concentrations. However, as particle size and shape affect the level of exhibited toxicity, direct comparisons of activity between studies are often difficult due to the different types of NP examined. One particularly promising NP substance is the organic biodegradable substance chitosan which is considered environmentally friendly. Chitosan has also been shown to stimulate plant growth and defence in addition to possessing antifungal activity. The lack of toxicological properties marks chitosan as having particular potential for fulfilling the regulatory requirements for environmental fate and ecotoxicology necessary for gaining approval as an authorised pesticide. Another distinct problem in comparing studies is the lack of a recognised standardised growth medium/media for determining nanomaterial toxicity. A growing body of evidence suggests that the *in vitro* toxicity of certain nanoparticles is highly influenced by the properties of the growth medium, such as its pH, salinity and components. These confounding factors will be discussed and their implications for comparing nanomaterial efficacy highlighted while also providing suggestions for improving characterisation of nanomaterial efficacy. Characterisation of nanomaterial efficacy *in vitro* is a critical step in determining which nanomaterials should be progressed for further testing in higher tier tests such as simulated use trials and field trials. The aim of this chapter is to draw attention to the limitations of *in vitro* characterisation and highlight how these techniques can be improved.

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

Keywords: Chitosan, Copper, Fungi, Growth medium, *In vitro*, Magnesium, Nanoparticles, Oomycetes, Phytopathogens, Silver, Zinc.

INTRODUCTION

Fungal and oomycete plant pathogens (phytopathogens) inflict substantial economic impacts on global agriculture each year [1]. The effects of fungal and oomycete phytopathogens can be devastating to certain crops resulting in restrictions to the supply of foodstuffs. As global production of food must rise in line with the increasing human population, control of such pathogens is of the utmost importance [2]. Typically, control of these phytopathogens is undertaken using synthetic pesticides whose mechanisms of action (MOA) target molecular pathways of pathogens such as signalling pathways, sterol synthesis and cell growth [3]. However, the resistance of fungal and oomycetes to such pesticides is continually evolving, requiring the development of novel control strategies [4]. One such potential novel solution could be the utilisation of a range of different substances in nanoparticle (NP) form as pesticides [5].

There has been significant interest in the antimicrobial properties of a range of NP substances for use in applications such as biocides, medical science and disinfectants, as well as in agriculture as Plant Protection Products (PPP) [5, 6]. Nanoparticulate substances, particularly those which are metal or metalloid based, generally exhibit broad-spectrum activity inducing damage to a range of cell organelles and molecular processes [7]. This broad-spectrum activity makes NPs particularly useful for use as general pesticides for controlling the broad range of fungal and oomycete pathogens typically associated with any particular crop.

The MOA of NPs and their resulting mode of action (MoA) in microbial species has been well characterised in a number of reviews [8, 9] and will only be summarised in this mini-review. In this review, the MOA refers to the specific biochemical mediators of toxicity such as ions and reactive oxygen species (ROS), while MoA refers to observed anatomical or functional changes in target organisms. Briefly, NPs have been shown to be highly reactive, possessing high surface energies, particularly for particles with diameters <30 nm [8]. The high surface energies associated with NP substances have been shown to induce a range of processes which can result in toxicity to cells and cellular processes [8, 9]. Nanoparticulate substances have been shown to inflict physical damage to microbial cell membranes/walls that they come into direct contact with, also in addition to generating ROS from their surface [8 - 10]. Certain metal-based NPs have also been shown to exhibit increased rates of ion dissolution, particularly smaller particles with more angular shapes [11]. Ions such as those released by nanoparticles of silver (AgNP) and copper (CuNP) have been shown to be directly toxic to microorganisms [9]. Released ions have also been shown to be a source of ROS which in turn inflict high levels of cellular toxicity [9]. The MOA and MoAs of each NP substance covered in this mini review are explored in more detail in later sections.

To date, numerous studies exploring the efficacy of nanomaterials for controlling fungal and oomycete phytopathogens have been published. To the authors' knowledge the range of published studies that examined the *in vitro* effects of NPs on fungal and oomycete phytopathogens included in this review is comprehensive, though we acknowledge some studies may be overlooked. The full list of studies included in this review and the pathogens they investigated can be found in Table **1**. Most often, in the *in vitro* studies, NPs are amended into a solid agar-based growth medium (AGM) which the fungi or oomycete are cultivated on. Alternatively, circular disks are saturated with an NP and are then placed on a solid growth medium to test antifungal activity *via* the disk diffusion method (DDM) or a well diffusion method (WDM) is used where aliquots of NP suspensions are deposited into "wells" bored into solid media These studies have identified a range of nanoparticulate molecules, elements and oxides that have the ability to inhibit the growth of pathogenic species *in vitro* and therefore may have potential to control diseases in the field. The most notable elements whose efficacy has shown promise in nano form are Ag, Cu and Zn, while the oxides MgO and ZnO have also shown significant promise. Nanoforms of the organic molecule chitosan have also been investigated for controlling certain pathogenic species *in vitro* and demonstrated promising efficacy. The range of fungal and oomycete plant pathogens whose growth has been inhibited by nanomaterials includes both soil-borne and foliar disease-causing species. The purpose of this chapter is to compare the studies performed investigating the efficacy of nanomaterials against plant pathogenic fungi and identify the common trends emerging from the research conducted to date.

Table 1. List of fungal and oomycete phytopathogens on which the NP substances have been tested *in vitro*.

Pathogen	Disease(s)	Host(s)	References
Alternaria alternata	Brown/Black/Leaf spot	*Citrus, Malus, Nicotiana, Pyrus, Solanum*	[27, 35, 36, 38, 43, 55, 63, 68]
Alternaria brassicicola	Black spot	*Brassica*	[32, 57, 68]
Alternaria solani	Early Blight	*Capsicum, Cucumis, Solanum*	[34, 62, 68]
Aspergillus flavus	Ear rot, Yellow mould	*Zea*	[25, 36, 51, 54, 56]
Aspergillus niger	Black mould	*Allium, Arachis, Vitis*	[25]

CHAPTER 10

Cosmetic and Medical Applications of Fungal Nanotechnology

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Abstract: Nanotechnology is the science of manipulating atoms and molecules in the nanoscale - 80,000 times smaller than the width of a human hair. Nanotechnology is a revolutionary technology that is being used in many fields all over the world as it finds applications in automobiles, electronics, material science, *etc*. Fungal nanotechnology has great prospects for developing new products with industrial, agricultural, medicinal, and consumer applications in a wide range of areas. Nanotechnology has applications in the field of cosmetics, which are known as nanocosmetics. Various types of nanomaterials are employed in cosmetic and medical applications *i.e.* inorganic nanoparticles, Silica (SiO_2), Carbon Black, Nano-Organic materials, Nano-Hydroxyapatite, Gold, and Silver Nanoparticles, Nanoliposomes, *etc*. NPs have been explored and identified as carriers for drug delivery. New drug delivery systems based on nanotechnology have been applied in the treatment of human diseases, such as cancer, diabetes, microbial infections, and gene therapy. The benefits of these treatments are that the drug is targeted to diseased cells, and its safety profile is enhanced by the reduced toxic side effects to normal cells. In general, NPs can be conjugated with different types of drugs to deliver bioactive compounds to the target site by various methods, such as the use of nanotubes, liposomes, quantum dots, nanopores, and dendrimers. It is employed in fuel cell applications that involve polymers in the proton exchange membrane, binder for the electrodes, and matrix for bipolar plates.

Keywords: Fungal Nanotechnology, Nanotechnology, Nanocosmetics, Nanoparticles, Nanosensors, Nanocosmaceuticals.

INTRODUCTION

Nanotechnology is the study of controlling particles and atoms inside the nanoscale - multiple times less than the width of an individual's hair. The world

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

commercial center for items that contain nanomaterials is anticipated to prevail in $2.6 trillion by 2015.

The utilization of nanotechnology has extended its boundaries across different surges of science, from gadgets to medication [1]. Nanotechnology holds colossal potential inside the analysis, treatment, and avoidance of COVID-19. Nanotechnology could help the battle against COVID-19 through various methodologies, such as staying away from viral tainting and shower by (a) plan of contamination safe individual defensive gear (PPE) to reinforce the security the wellbeing of medical services laborers and improvement of viable antiviral sanitizers and surface coatings, which are prepared to inactivate the infection and forestall its spread; (b) plan of exceptionally explicit and delicate nano-based sensors to rapidly recognize the disease or immunological reaction; (c) advancement of most recent medications, with upgraded movement, diminished poisonousness and supported delivery, additionally as tissue-focus, for example, to the lungs; and (d) advancement of a nano-based inoculation to zest up humoral and cell safe reactions [2].

Nanotechnology is a progressive innovation that is being utilized in many fields all around the world as it discovers applications in autos, gadgets, material science and so on. Contagious nanotechnology has extraordinary possibilities for growing new items with modern, agrarian, restorative, and customer applications in a wide scope of regions. The natural and clinical examination local area of the world is focussing on how they can make tranquilizes less expensive, more compelling, how could decrease results of medications how might deal with infections like a malignancy in the best manner, how could foster new devices and gadgets which can diminish agony and draining which is normal during medical procedures and tasks. The fields of synthetic designing, agri-food, organic chemistry, drugs, diagnostics, and clinical gadget advancement all utilize contagious items, with parasitic nanomaterials as of now utilized in applications going from drug improvement to the food business and agrarian biotechnology. Contagious specialists are climate agreeable, clean, non-toxic specialists, for the metal nanoparticles combination and utilize both intracellular and extracellular techniques [3]. A few nanoparticles, for example, gold nanoparticles, silver nanoparticles, different nanoparticles integrated by numerous universal parasitic species, for example, *Trichoderma*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Pleurotus* and *Aspergillus*, *Beauveriabassiana*, and so on the effortlessness of increasing and downstream handling and the presence of contagious mycelia which manage the cost of an expanded surface region give key benefits [4]. Understanding the diversity of parasites in arranged environments, as well as their interactions with various microorganisms, creatures, and plants, enables genuine and imaginative mechanical turns of events and the application of metal nanoparticles in a variety of fields, including farming, catalysis, biomedical biosensors, and beauty care products.

NANOTECHNOLOGY IN COSMETICS

Nanotechnology has applications in the field of beauty care products, which are known as nanocosmetics. This far and wide impact of nanotechnology in the restorative enterprises is because of the expanded properties accomplished by the particles at the nano level including straightforwardness, shading, solvency, and so on the various sorts of nanomaterials worked in beauty care products incorporate nanosomes, liposomes, fullerenes, strong lipid nanoparticles and so on through the utilization of nanotechnology applications in beauty care products we can make corrective items which helps in eliminating wrinkles and dim spots and has brilliance impact with sunscreen, moisturizes the skin and firms, lights up the sensitive skin around the eyes, supports collagen union, skin fix.

As of late, issues over the wellbeing of such nanocosmetics were raised and have constrained the corrective enterprises to restrict the utilization of nanotechnology in beauty care products and for installing laws to go through an undeniable security appraisal before they go into the market. The makeup business was among quick to carry out nanotechnological standards in item improvement. More than 1,000 enrolled nanotechnology-put together items concerning the worldwide market in 2009, over 13% were named items for corrective use [5].

The uses of nanotechnology and nanomaterials are found in numerous corrective items, including creams, hair care items, makeup, and sunscreen. In cosmetics and skin health management, there are two primary uses for nanocosmetics:

1. Nanoparticles in beauty care products as UV channels.
2. Nanoparticles in beauty care products as medication conveyance specialists.

In the broader aspect of nanotechnology, NPs can be divided into two groups:-

Chitin and its deacetylated subsidiary chitosan are of extraordinary interest to the cosmeceutical business because of their exceptional biological and mechanical properties. A chitin nanofibril is an illustration of a nanocrystal got from the scavenger exoskeleton, disposing of carbonate and protein segments while as yet being viewed as protected to utilize. Chitin nanofibrils in emulsions can produce the development of a hygroscopic sub-atomic film that dials backwater, vanishing and adds to skin hydration .

CHAPTER 11

Nanobiotechnological Strategies for Detection of Fungi and Mycotoxins in Food and Feed

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Abstract: Mycotoxins are poisonous compounds that are produced by toxigenic fungi as secondary metabolites. Their production can occur at any stage of food and feed supply, including harvesting, storage, processing and distribution, contaminating a plethora of foodstuffs. As mycotoxins exert their toxic properties at a very low level, usually at μg/kg level, their early and fast detection in food materials is necessary. The early detection of mycotoxins and fungi contamination could pose the elimination or reduction of possible threats associated with the consumption of mycotoxin-contaminated food. Contamination of food with mycotoxins can be prevented by monitoring and control at various critical stages of the food chain at the pre-and post-harvest stage. Given the widespread use and rapid development of nanotechnology in a variety of fields, it is considered that the application of many nanomaterials in the detection of mycotoxins will be a pioneering strategy. Except for conventional methods, such as gas chromatography and high-performance liquid chromatography coupled with mass spectrometry or other detectors, various nanotechnological approaches are used for the detection of fungi and mycotoxins. Nanobiosensors, nanoparticles, nanowires, nanorods, and nanodiagnostic kits are used for the rapid detection of mycotoxin in food analysis. In addition, the electronic nose and electronic tongue are highly helpful in detecting a variety of mycotoxins and mycotoxin-producing fungi in food and feed. The main purpose of this chapter is to describe the role of nanobiotechnology and nanomaterials in the detection of fungi and mycotoxins in food and animal feed.

Keywords: Detection, Electronic nose, Electronic tongue, Food quality, Fungi, Mycotoxins, Nanobiotechnology, Nanobiosensors, Nanodiagnostic kits, Nanoparticles, Nanorods, Nanowires.

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

INTRODUCTION

Mycotoxins are a group of toxic fungal metabolites found in a wide range of food and feed products worldwide, with carcinogenic, genotoxic, teratogenic, nephrotoxic, and hepatotoxic effects [1, 2]. Although some fungi are beneficial to man, producing useful metabolites that contain pharmacologically active drugs for treating man and animals, some are responsible for human infections of the eyes, nails, hair, and skin, some fungi are pathogenic and cause extensive damage and agricultural losses to many crops, and some species produce mycotoxins [3]. Fungal infection is a huge threat to the economy due to reduced input and exports of foods and feedstuff affected by fungal growth [4]. Contamination of agricultural products with mycotoxins is a major problem mainly in tropical and subtropical regions, where climate and poor storage conditions favour the growth of fungi and the production of mycotoxins [5]. Many agricultural products such as nuts, fresh and dried fruits and vegetables, cereals such as maize, rice, and wheat, flours, liquids such as wine, grape juice and beer, milk and dairy products, spices and herbs, coffee and cocoa, and feed can be contaminated with mycotoxins at all stages of the food and feed chain, both pre-and post-harvest [2, 6, 7]. The main producers of mycotoxins are the fungi of the genera of *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria* [8].

The major classes of mycotoxins that are of the greatest agroeconomic importance are aflatoxins, ochratoxins, fumonisins, trichothecenes, emerging Fusarium mycotoxins, enniatins, ergot alkaloids, Alternaria toxins, and patulin, causing diseases both in humans and animals, known as mycotoxicosis [2 - 9]. To date, about 500 mycotoxins have been identified, while another 1000 are expected to be identified. Concerning so-called masked mycotoxins, the risk is extremely high, as there is no established method for their determination [10] and as their reconvert to their native form in the digestive tract [11].

Among all mycotoxins, aflatoxins are extremely dangerous to the health of humans and animals, as showing carcinogenic, mutagenic, teratogenic and immunosuppressive actions [12]. Consumption of mycotoxins contaminated food and feed by humans and animals respectively cause chronic or acute toxicity, a disease commonly attributed to the term mycotoxicosis [13] and even death [14]. Cardiotoxicity, central nervous system disorders, gastrointestinal tract damage, nephrotoxicity, and hepatotoxicity are some negative health impacts that are caused by mycotoxins [15]. The US Centers for Disease Control (CDC) estimates that more than 4.5 billion people are chronically exposed to high levels of contaminants by mycotoxins food [16].

The European Union (EU), the US Food and Drug Administration (FDA) and many authorities from other countries have established regulatory guidelines and maximum levels for many mycotoxins of major concern including aflatoxins (AFs) such as aflatoxin AFB1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) and aflatoxin M1 (AFM1), ochratoxin A (OTA), deoxynivalenol (DON), fumonisins (FBs), T-2 and HT-2 toxins (T-2, HT-2), zearalenone (ZEN) and patulin (PAT), for both imports and exports of contaminated food and feed [17, 18].

Numerous efforts have been made by many researchers to develop methods for detecting mycotoxins in food and feed, with many of these methods focusing primarily on chromatography and immunology [19]. Conventional techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) in combination with different detectors like ultraviolet (UV), fluorescence detector (FLD) or diode array (DAD), liquid chromatography coupled with mass spectrometry (LC–MS), liquid chromatography-tandem mass spectrometry (LC–MS/MS) and gas chromatography-tandem mass spectrometry (GC–MS/MS) are the main chromatographic techniques, which are used for detection of mycotoxins. As it concerns immunochemical techniques, enzyme-linked immunosorbent assay (ELISA) and lateral-flow devices (LFDs) are the main representatives [8]. Prerequisites are required for the application of all these techniques, such as sampling, extraction, and cleanup methods [20]. Moreover, all the above-mentioned techniques are very accurate, but they have some limitations that are associated with these techniques, such as the high cost, the extensive and lengthy sample preparation, which takes a long time, and the need for bulky instruments and skilled personnel [21], limiting their use in the feed and food industries [22]. The development of new, simple, robust, reliable, inexpensive and fast techniques by researchers is the need of the hour [23, 24].

As it concerns, the conventional method for analyzing the load of toxicogenic fungi is based on the plate counting method which is done by plating dilutions on a single culture plate and then recording colonies [25]. In addition, regular fungal identification is mainly based on phenotypic methods, which often require expertise in morphological analysis and the result is always expressed in the order of magnitude such as 1×10^n CFU/g [26]. Moreover, DNA-based detection, molecular assays, like, polymerase chain reaction (PCR) or quantitative PCR (qPCR), enzyme-linked immunosorbent assay, is also used in early detection for fungal spoilage organisms [27].

Nowadays more sophisticated techniques such as spectroscopy techniques namely Raman spectroscopy, Fourier transform infrared (FTIR) spectroscopy and hyperspectral imaging (HSI) have been used by researchers for detecting

CHAPTER 12

Early Detection of Crop Fungal Pathogens for Disease Management using DNA and Nanotechnology Based Diagnostics

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Abstract: Virulent fungal plant pathogens are a serious threat to crop productivity and are considered a major limitation to food security worldwide. To meet these challenges, pathogen detection is crucial for taking appropriate measures to curb yield losses. Disease diagnosis at an early stage is one of the best strategies for crop protection. Earlier, traditional methods were used to diagnose and manage fungal diseases, which included visual scouting of the disease symptoms and spray of fungicides. The utility of immunoassays for early detection and precise identity has been appreciably stepped forward following the improvement of enzyme-connected immunosorbent assay (ELISA) and monoclonal antibodies. Nucleic acid-based diagnostic techniques have turnout to be the preferred type because of their greater speed, specificity, sensitivity, reliability, and reproducibility. The biosensor eliminates the need of sample preparation and can be used for on-site detection of fungal pathogens at latent infection stages so that preventive measures can be taken. Currently, multiple human and animal diseases have been detected with the help of biosensors. However, reports on plant pathogen detection using biosensors are still in infancy. Despite many applications of antibodies, there are also multiple drawbacks, including high cost, low physical and chemical stability, and the ethical issues associated with their use. Now, DNA based biosensors are gaining popularity because of their sensitive and precise detection of DNA target sequences. Immunological and DNA-based techniques combined with nanotechnology offer highly sensitive and selective gel-free detection methods, and the lab-on-chip (LOC) feature of biosensors makes them a very reliable tool in crop protection.

Keywords: Biosensors, Diagnostic techniques, DNA based biosensors, Fungal Pathogen, Immunosensor, Nanotechnology.

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INTRODUCTION

Global food security is the major concern of the world due to the growing population in recent years. Agricultural efficiency and yields are limited worldwide by both abiotic (non-biological) and biotic (biological) factors such as water shortages, salinity, drought, heat stress, and environmental degradation, vulnerability to weather-related disasters and plant pathogen, insect pest attack. Plant diseases caused by pathogen attack may lead to devastating effect on social life, economy and ecology globally, harming biodiversity as well. Approximately 20% to 40% of losses in major agricultural crops are due to biotic stress *i.e.* by plant pathogen attack during sowing, germination, growth and development, which pose a wide spectrum of challenges to agricultural scientists in crop production. These are considered to be the major obstacle to global food security [1]. The term “pathogen” is defined as the infectious biological entity that could be bacteria, viruses, fungi, nematodes or any other microorganism that can infect human beings, animals and plants and lead to the chain of infections, diseases and symptoms and make them sick [2]. Plant pathogens that cause disease in plants are also known as phytopathogens. Most of the plant pathogens cause harm to plants but there are some trans-kingdom pathogens that can make immunocompromised people sick [3]. New, old and emerging plant infectious diseases are the main reason for low agricultural productivity. Table **1** shows the fungal pathogens which adversely affect crop productivity. The higher degree of transmission, occurrence and extremity of the pathogens makes them harmful to the world food supply [4]. Worldwide, pathogens and pests are causing losses to the tune of 10% to 28% in wheat, 25% to 41% in rice, 20% to 41% in maize, 8% to 21% in potato, and 11% to 32% in soybean [5]. Usually, a number of plant pathogenic organisms show their symptoms after some days or months. Thus, this helps in routine laboratory diagnosis and pathogen detection. But nowadays, early as well as fast detection for pathogen become necessary for several reasons, like climate change, globalization, increased human mobility, international trade, pesticides resistant pathogens. Due to these reasons, the timely spray for eradication of pathogen becomes no longer endurable for farmers. The development of the agriculture sector is only possible by using modern technologies with the minimum damage to agroecology [6]. There are enormous challenges for plant disease detection, research and practical applications [7]. Moreover, there is an urgent need to take preventive measures to protect crops that are economically important and for this, on-site diagnostic methods are required. The on-site diagnosis will help in developing proper field operable strategies for early plant pathogen control that will prevent the spread of disease and control by assisting in better understanding of disease epidemiology and also improving the design cultivar of choice [8].

Table 1. List of the plant fungal pathogens that cause serious yield losses in crops.

Crops	Fungal Pathogen	Common Name
Grasses and Cereals like Barley	*Blumeria graminis*	Powdery mildew
Cereals	*Fusarium graminearum*	Blight, Root and Crown rot and Head blight on small grain Cereals
Wheat	*Mycosphaerella graminicola*	Septoria tritici blotch
	Pythium ultimum	Damping off
	Puccinia spp.	Rust
	Melamp soralini	Rust
	Septoria tritici	Blotch
Barley	*Puccinia spp.*	Rust
Rice	*Magnaporthe oryzae*	Blast
Maize, Teosinte	*Ustilago maydis*	Smut
Corn	*Pythium ultimum*	Damping off
Tomato	*Fusarium oxysporum*	Wilt
	Phytophthora infestans	Late blight
	Phytophthora parasitica	Rot disease of root and stem
Potato	*Phytophthora infestans*	Late blight
	Pythium ultimum	Damping off
Soybean	*Pythium ultimum*	Damping off
Cabbage, Kale, Lettuce	*Albugo candida*	White rust
Linseed	*Melampsoralini*	Rust
Peppers	*Phytophthora capsici*	Blight, root rot and stem rot
Eggplant	*Phytophthora parasitica*	Rot disease of root and stem
Tobacco	*Phytophthora parasitica*	Rot disease of root and stem
	Fusarium oxysporum	Wilt
Legumes	*Fusarium oxysporum*	Wilt
Cucurbits	*Fusarium oxysporum*	Wilt
Sugar beet	*Albugo candida*	White rust
Woody and herbaceous plants	*Colletotrichum spp.*	Anthracnose
Grapes	*Botrytis cinerea*	Grey mould
	Plasmopara viticola	Downy mildew
Pineapple, Papaya	*Phytophthora parasitica*	Rot disease of root and stem

CHAPTER 13

Potential Role of Nanotechnology in the Wood Industry to Develop Resistance against Fungi

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Abstract: Wood properties can be changed using nanomaterials that can penetrate deeply into the wood substrate. This capacity of nano-based materials can be utilized in changing wood properties in a way that is very effective in their long-term use. Nanotechnology can certainly change the future of the wood industry by increasing the functional life of wood products as well as usability under various conditions. But its full potential to make wood resistant against fungi has still not been explored. Research is underway but there is still a long way to go. Studies carried out on the use of nanoparticles have clearly shown the negative impact of nanoparticles on human health as well as on the environment. This issue needs to be addressed.

Keywords: Biocides, Nanotechnology, Self-cleaning surface, Scratch resistance, Termite resistance, Wood industry.

INTRODUCTION

Wood is an important plant product required for the very existence of mankind. Wood is a source of fuel and myriad of the products obtained/manufactured, which is derived from the forests partly or wholly. Durability of wood refers to its natural ability to resist the attack by fungi, bacteria and insects. Wood as such and its different forms such as seasoned lumber, logs, *etc*. are susceptible to the attack by different types of pathogens causing immense loss to the wood industry. In order to protect the wood against the damaging effects of pathogens, it is either seasoned or treated with various preservatives. The important chemical preservatives are copper naphthenate, zinc chloride, creosote, Wolman salts, pentachlorophenol or compounds of chromium, mercury and arsenic. To increase the durability of wood, generally two methods are used (i) protective coating with weather resistant paints, varnishes or lacquers, and (ii) immersing woods in open tanks, or spraying or injecting them with chemicals [1].

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In nature, the decay of wood by fungi is an important ecological process. The Xylophagous fungi are able to decompose wood and release nutrients locked up in wood back in the soil. The wood-rotting fungi are able to degrade various proportions of cellulose and lignin of the wood leading to their classification into categories such as Brown rot, White rot or Soft rot (Fig. **1**). The brown rot fungi are known to break down cellulose and hemicellulose that are the basic cell wall components [2]. The white rot fungi are known to attack and break down lignin present in the secondary wall which results in a white coloured decaying wood. These fungi are known to produce the laccase enzyme, which is involved in lignin degradation. Soft rot fungi, on the other hand, produce cellulase enzyme, which breaks down wood and makes it hollow and spongy from inside [3]. Understanding the biology and ecology of these fungi becomes important to develop strategies against them.

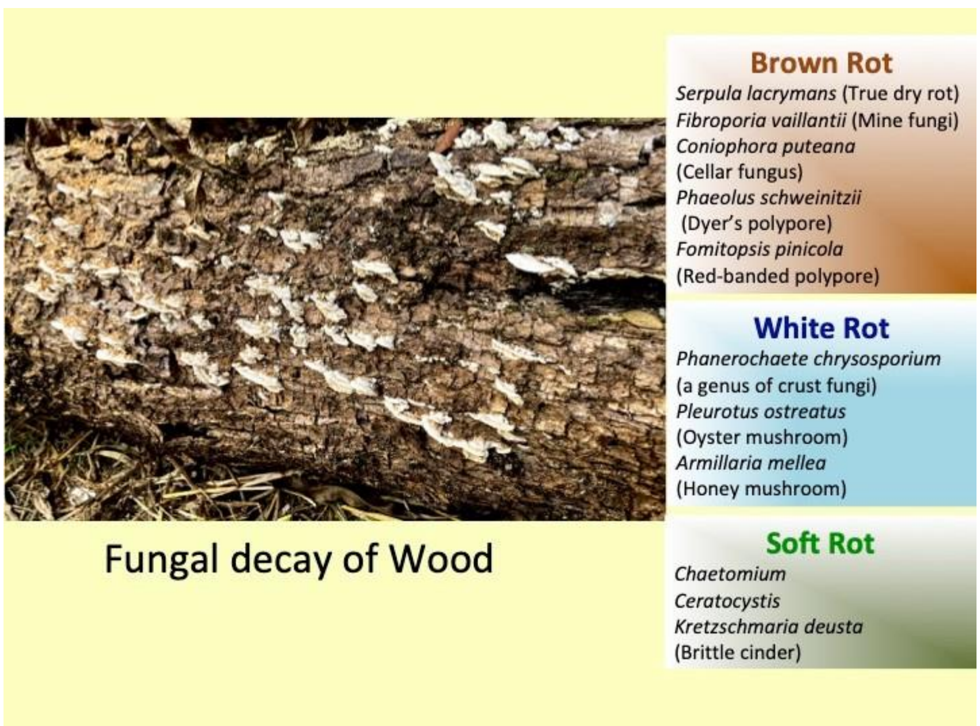

Fig. (1). Different genera of fungi responsible for the decay of wood. (Photo: by author).

Nanotechnology can play a significant role in wood preservation (Fig. **2**) [4, 5]. It is an emerging field that utilizes nanoparticles ranging in size from 1 to 100 nm. Through nanotechnology, nanosized wood preservatives e.g. copper, zinc oxide and silver can be prepared. These can then be applied to the wood directly *via* vacuum pressure treatment. Such treatment increases the time for which preservative is retained in the wood and thus increases its durability [6, 7].

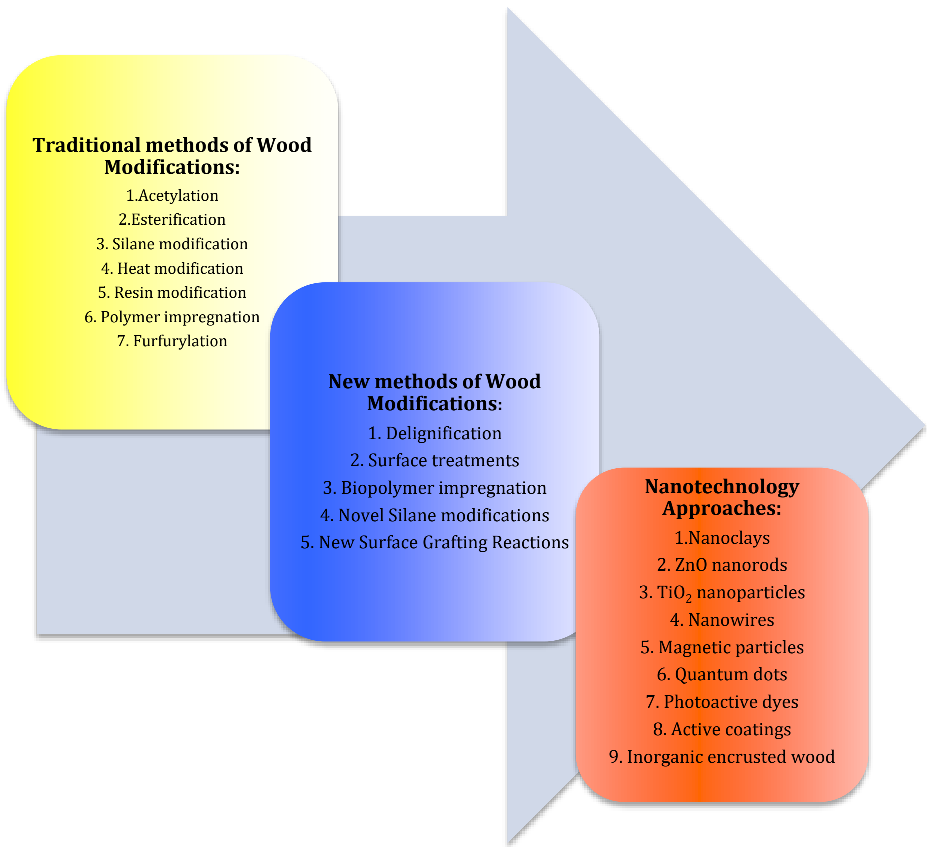

Fig. (2). Different methods of wood modification. (After Spear *et al.* 2021) [4].

The other methods of protecting the wood against fungi include the use of nanomaterials to modify the surface of the wood to increase its resistance against weathering [8, 9], and different nanocarriers can be used to encapsulate hydrophobic biocides to improve water dispensability [10]. Types of polymeric nanocarriers are shown in Fig. (**3**) [11]. In spite of these proposed applications of nanotechnology in the wood industry, the potential risks associated with nanoparticles must be assessed critically [12].

CHAPTER 14

Nano-Fungicides: Synthesis and Applications

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Abstract: Demand for food, fibre and medicines has been boosted tremendously for the explosive population, which has certainly built pressure on the agriculture-based sector to meet the requirements by various means. Nano-fungicides are fungicidal formulation that contains fungicide particle size 10^{-9}. These nano-fungicides contain antimicrobial properties, which could be utilized against plant pathogens such as fungi and bacteria as a potent pesticide. Nanoparticles of fungicidal properties can be synthesized using different metals *via*. copper, silver, *etc*. Recent reports suggest that nanoparticles can also be synthesized using biological means such as fungi which pose effective fungicidal actions. Nanopesticides have their application in various areas such as agriculture, food, medical industries, storage packaging of food, *etc*. The present chapter will light upon the types and methods of nanoparticle synthesis and their applications. Categories of nano pesticides based on their nature application and source of synthesis will also be covered. Inventions in nano pesticides could lead us to less dependence upon conventional chemical pesticides which have adverse effects on climate, animal and human health.

Keywords: Agriculture industry, Disease management, Nanoparticles, Nano-fungicides, Synthesis.

INTRODUCTION

In recent times nanotechnology is one of the vital approaches to detect nano-substances/particles, which has found innumerable applications in various fields such as, agriculture, pharmaceutical and biomedical sciences. Changing the environment has led to several outbreaks of infectious diseases among plants and humans which are caused by different pathogenic bacteria, fungi and viruses. These pathogens have also developed resistance to antibiotics, fungicides and virucides. Therefore, chemical industries and researchers are searching for new effective chemicals.

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Various natural and artificial methods for the management and protection of plants from these diseases have been applied so far; among them, a method for plant disease management, use of pesticides is the most prevalent. Here, nano-particle based fungicides and antimicrobial compounds have the potential to be offered a promising path for sustainable plant disease management. Nanoparticles (NPs) of metals contain some suppressive properties against some foliar and soil-borne fungi called nano-fungicides. Nano fungicides are mainly formed by metals such as (Cu-NPs, CuO-NPs), silver (Ag-NPs) and zinc (ZnO-NPs). The average nano-particles size ranging from 1-100 nm are suitable to fall under nanoparticles [1]. In recent years, the environmental damage caused by excessive and indiscriminate use of pesticides has been widely discussed; therefore, scientists in the agricultural field are searching for alternative measures against pesticides, where the use of nano-fungicides has emerged as an eco-friendly, effective and less pollutant option. The current chapter describes recently identified and applied metal-based nano-pesticides and their synthesis.

COPPER-BASED NANO-FUNGICIDES

Copper-based chemicals are well-proven antimicrobials in agriculture since the discovery of synthetic fungicides. Copper is an essential trace element with well understood biological roles in all living organisms, including plants, microorganisms or animals, when present in trace amounts. Further, copper-based chemical formulations have been reported to be one of the first fungicides used for plant disease management [2]. In an *in vitro* study of copper nanoparticles (CuNPs) on plant pathogenic fungi, oomycete, bacteria, and beneficial microbes *Trichoderma harzianum* and *Rhizobium spp*., along with wheat seeds, were conducted [3]. This study showed that CuNPs with non-nano copper-like copper oxychloride (CoC) at 50 mg/L concentration effectively inhibit 76% of *Phytophthora cinnamomi*. Copper nano fungicidal assessment on *Botrytis cinerea* suggested that maximum inhibition of mycelium is obtained in EC50 value range of 162 and 310 µg/mL. Copper nanoparticles were most effective in the form of $CuSO_4$ [4]. Copper nano-fungicides were also tested effectively against *Aspergillus flavus, Aspergillus niger and Candida albicans* [5]. Interestingly this study showed the non-biocidal effect of CuNPs on beneficial microbes, which suggests potential utilization as part of an integrated pest management strategy in the agri-ecosystem.

SILVER-BASED NANO-FUNGICIDES

As an alternative to chemically manufactured fungicides, the use of silver nanoparticles as antimicrobial or pesticide agents has become more common, as their technological advances make production more economical [6]. Silver nano-fungicidal assessment on fungi showed inhibition of fungal mycelium exclusively against *B.Gray*mould fungi *B. cinerea* [4]. Another composition of silver nano-particle was suggested where nano-sized silica-silver consisted of nano-silver combined with silica molecules and water-soluble polymer, prepared by exposing a solution including silver salt, silicate and water-soluble polymer to radioactive rays [7]. This study demonstrated that mentioned composition is more effective than Ag-NPs alone as it shows that silver silica nanoparticles can inhibit phytopathogenic fungi at 3 ppm whereas Ag-NPs alone inhibition can range from 10 ppm to 100 ppm. This has even reported inhibition of powdery mildew of pumpkin at 0.3 ppm. Fungicidal activity of copper nanoparticles was assessed on PDA (Potato Dextrose Agar) media against various fungi above fungi including *Alternaria alternata (Alternaria* leaf blight*) A. brassicicola* (Blackspot) *Cladosporium*, Leaf spot fungi *Corynespora,* scab fungi *Clados porium cucumerinum,* Anthracnosepathogen *Glomerellacingulata,* root rot fungi *Pythium aphanidermatum* and *Fusarium* spp., these fungi were significantly inhibited at 100 ppm concentration [8]. Similarly, nanoparticles at 100 ppm were also reported effective for pepper anthracnose pathogen caused by *Colletotrichum* species [9].

ZINC BASED NANO-FUNGICIDES

Zinc nano fungicidal assessment shows that it exhibits fungicidal activity in form of ZnO-NPs and in EC50 range of 235 and 848 µg/mL. ZnO-NPs were more effective than $ZnSO_4$ against fungi such as *B. cinerea, Alternaria alternata and Moniliniafructicola* [4]. Another study demonstrated that ZnO NPs are most effective against post-harvest fungi *viz*. *Botrytis cinerea* and *Penicillium expansum*. It recorded maximum activity at 3 mmol l^{-1} and both fungi were significantly inhibited but *P. expansum* showed more sensitivity to ZnO NPs. ZnO deform the fungal hyphae and prevent the formation of conidiophore and conidia [10].

A study on lentil pathogens *Alternaria alternata*, *Fusarium oxysporum* f. sp. *lentis*, *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas syringae*pv. *Syringae* and *Meloidogyne incognita* conducted which demonstrates Spray of ZnO NPs reduces nematode multiplication, wilt, leaf spot and blight disease severity indices [11].

GOLD-BASED NANO-FUNGICIDES

The novel metal gold is also being studied for its potential use as nano-fungicides (AuNPs). A study on the green synthesis of gold nanoparticles using 1 mM gold chloride solution with leaf extract of *Annona muricata* reveal that the synthesized gold nanoparticles exhibited good antimicrobial activity depending on the

CHAPTER 15

Role of Fungi in Biofuel Production Chain

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Abstract: The demand of fuels as a source of energy for various operations is increasing daily. This has led to increased demand of fossil fuels, particularly by transportation and industrial sectors. There are multiple problems related to conventional fossil fuels like firstly, they are non-renewable resources with limited reserves. Secondly, fossil fuels pose serious environmental and health issues. Fossil fuels are one of the leading sources of emission of atmospheric greenhouse gases (GHG), resulting in global warming and thus climate change. These limitations and adverse effects of the use of fossil fuels have warranted scientists and policymakers to look for renewable and greener alternatives such as biofuels. Based on the type of feedstock used, biofuels are classified as first-generation, second-generation and third-generation. First-generation biofuels are based on edible resources which are already scanty. This has led to increased interest in second and third-generation biofuels. The agricultural waste and inedible crops constituting lignocellulosic materials are important second-generation biofuel feedstocks. The second-generation feedstocks can be a great alternative to conventional fossil fuels, but there are a few limitations, such as the cost and efficiency of production. Currently, scientists are looking at the role of fungi and utilization of various fungal enzymes in the hydrolysis of the lignocellulosic substrates for efficient and cost-effective production of biofuels. Nanomaterials have the ability for the better utilization of enzymes, biofuels, biodiesels and other microbial fuels. Therefore, nanotechnology can be utilized to address the challenges through various mechanisms and processes. This chapter is an attempt to focus on the role of fungi and fungal enzymes for better utilization of feedstock and sustainable production of renewable, cost-effective, environment-friendly biofuels.

Keywords: Biofuels, Fossil fuels, Fungi, Fungal enzymes, Lignocellulosic materials.

INTRODUCTION

There is increased demand of energy due to fast growing world's population along with the unprecedented speed of industrialization leading to energy crises. Guo *et al.* 2015, has predicted increased world energy demand to 8.6×10^{20} J in

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Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

2040 and was predicted 6.6 × 10^{20} J for the year 2020 [1]. This will lead to increased fossil fuel utilisation particularly by industry and transportation sector. There are multiple problems which are related to the utilization of conventional fossil fuels to meet this increased demand. First of all, they are non-renewable resources with limited reserves, which are depleting at a very fast rate. This has also led to increased crude oil prices globally. Secondly, utilization of fossil fuel is one of the leading factors contributing to global warming, environmental pollution and health issues [2]. The increase in the levels of greenhouse gases (GHG) as a consequence of burning of fossil fuels will lead to global warming and, therefore climate change [3, 4]. Consequently, there is an immediate need to shift the focus from fossil fuels to alternative renewable, environmentally friendly sources of energy. Over the past few decades, policy makers and scientists have been exploring ways and means to utilise agricultural crops and wastes as a source (feedstock) for fuel. Biofuels or agrofuel are mainly derived from biomass or biological waste such as agricultural waste. Nanomaterials/Nanoparticles are emerging technology for efficient and speeding up biofuel production and their role can't be ignored in biofuel production now and in future. Nanotechnology can help us in producing biofuel at a lesser cost than conventional methods. These biofuels have emerged as one of the potential renewable and environment friendly alternatives to conventional fossil fuels.

First-generation: these are based on edible plants, specifically starch or oilseeds crops such as corn, potato, wheat, rapeseed, soybean, sunflower [5].

Second-generation: these are based on inedible crops or agricultural wastes mainly consisting of lignocellulosic materials [5].

Third-generation: these are based on the use of different types microorganisms like microalgae for the production of different types of biofuels like bioethanol and biodiesel [6].

CLASSIFICATION OF BIOFUELS BASED ON FEEDSTOCK TYPE

Biofuels can be classified into three generations based on the type of feedstock starting material used (Fig. **1**).

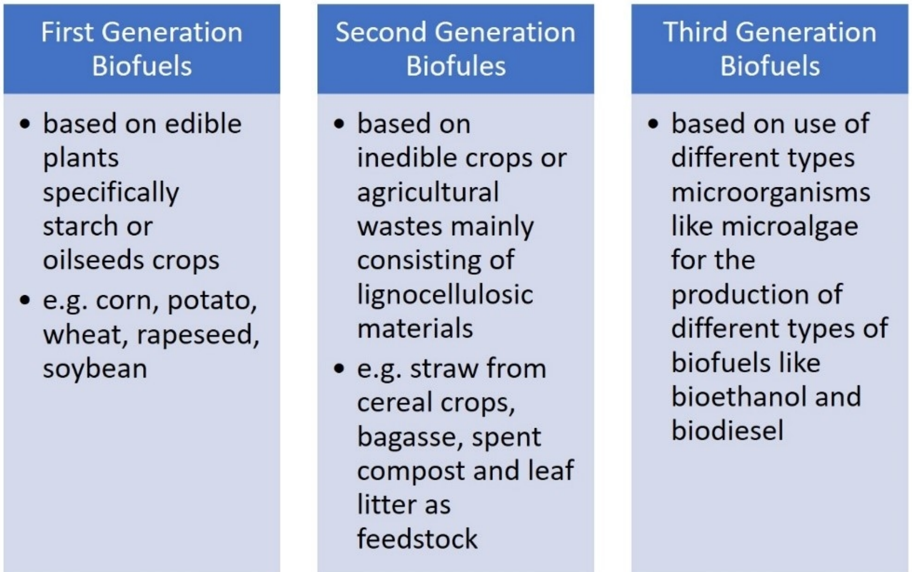

Fig. (1). Classification of biofuels on the basis of type of feedstocks.

Biofuels can be further classified into three forms based on the physical state of biofuels (Fig. **2**) [1, 5]:

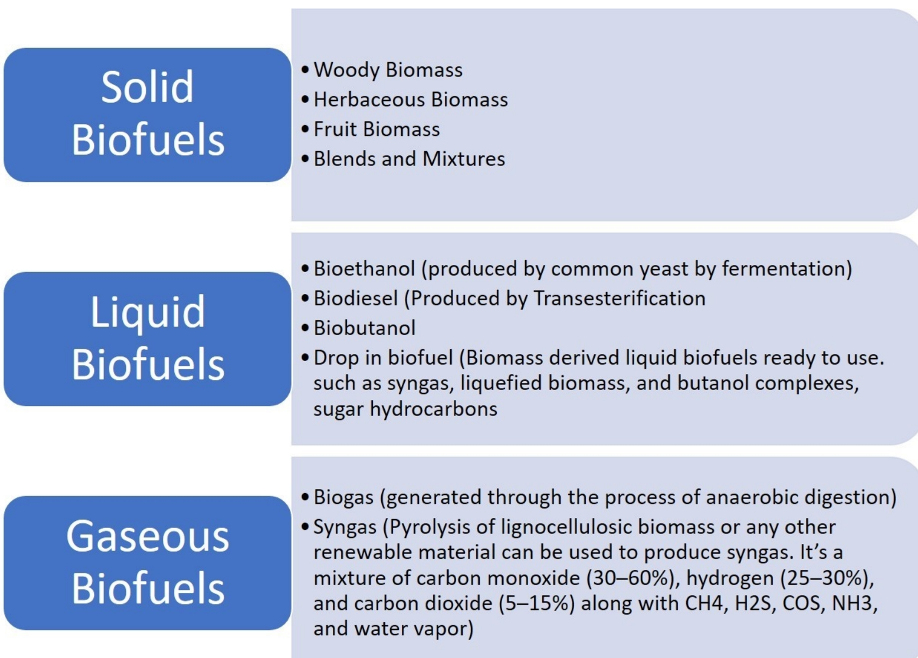

Fig. (2). Classification of biofuels based on their physical state.

SUBJECT INDEX

A

Savita, Anju Srivastava, Reena Jain & Pratap Kumar Pati (Eds.)

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

X

Y

Z

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